GENE-BY-DIET INTERACTIONS AND OBESITY AMONG YUP'IK PEOPLE LIVING IN SOUTHWEST ALASKA

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Dominick Lemas, BS

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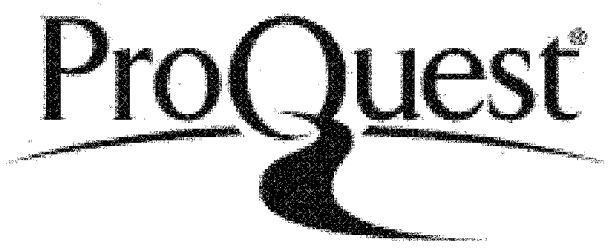

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By

Dominick Lemas

RECOMMENDED:

Hemant Kumar Tiwari

Advisory Committee Chair

Chair, Department of Chemistry and Biochemistry

APPROVED:

Dean, College of Natural Science and Mathematics

Dean of the Graduate School

11/29/2012

Date

ABSTRACT

BACKGROUND: Molecular approaches have expedited the discovery of human obesity genes, however the heritability explained by these loci remains low (<2%). Gene-by-environment interactions may partially account for the "missing heritability" attributed to variation in obesity phenotypes. OBJECTIVE: The specific aims of this dissertation were to (i) identify genetic polymorphisms associated with obesity-related phenotypes in Yup'ik people and (ii) evaluate how n-3 polyunsaturated fatty acid (n-3 PUFA) intake modifies associations between genetic polymorphisms and obesity-related phenotypes in a population with widely varying intake of n-3 PUFAs. APPROACH: We genotyped genetic polymorphisms in (1) candidate genes with a strong physiological role in obesity pathophysiology; (2) candidate genes identified in obesity whole-genome linkage studies that were regulated by n-3 PUFAs; and (3) candidate genes reproducibly implicated in obesity genome-wide association studies (GWAS). DATA & ANALYSES: We used Center for Alaska Native Health Research (CANHR) data collected between 2001 and 2008. We estimated dietary intake of n-3 PUFA using nitrogen stable isotope ratios ($\delta^{15}N$) of red blood cells (RBC) and obesity-related phenotypes were obtained by trained staff. Genotype-phenotype analyses used generalized linear models that accounted for familial correlations. RESULTS: Our analyses of candidate genes based on physiology revealed a polymorphism called P479L in carnitine palmitoyltransferase 1A (*CPT1A)* that was associated with elevated fasting HDL-cholesterol and all obesity phenotypes. Our investigation of candidate genes that are regulated by n-3 PUFAs and implicated in obesity whole-genome linkage studies demonstrate that polymorphisms in stearoyl CoA desaturase (*SCD)* and steroyl regulatory element binding protein *(SLC2A4)* were associated with obesity-related phenotypes; however n-3 PUFA intake did not modify associations between *SCD* and *SLC2A4* polymorphisms and obesity phenotypes. Finally, our investigation of candidate genes reproducibly implicated in obesity GWAS demonstrated that genetic predisposition to obesity is associated with adiposity and that interactions with n-3

PUFA intake accounted for more than twice the phenotypic variation in adiposity. CONCLUSION: Taken together, results from this dissertation suggest that selecting candidate genes based on large-scale genomic analyses, such as linkage analyses and GWAS, has the potential to identify gene-by-environment interactions that partially account for the "missing heritability" attributed to obesity.

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INTRODUCTION

1.1 OBESITY EPIDEMIC

Obesity is characterized by excess body fat and increases the risk of developing type 2 diabetes (T2D), stroke, cancer, and other chronic diseases (1). Current estimates indicate that nearly 70% of adults in the United States are overweight and more than half of these individuals are obese (2). The development of obesity occurs in response to joint actions of multiple genetic and environmental factors that increase the risk of accumulating excess body fat (3). Although moderate levels of weight loss (~10% of body weight) have been shown alleviate many of the major health risks attributed to obesity (4), a large number of overweight and obese individuals are unsuccessful with long-term weight management (5). Given the increasing public health burden associated with obesity and the difficulty achieving and maintaining weight loss (6–8), understanding the genetic and environmental factors that influence obesity has implications for designing more effective therapies and personalized interventions.

1.2 DISSECTING THE HERITABILITY OF OBESITY

Studies of large families, twins, sibling pairs, and adopted individuals have been used to estimate the heritability of body mass index (BMI kg/m^2) to be between 40 and 70% (9, 10). Heritability is a measure of the phenotypic variance in a population that is attributable to genetic factors (11). For approximately 20 years, molecular approaches attempting to identify genetic factors that explain the high heritability of obesity were limited to either candidate genes studies or linkage analyses (12). Candidate gene approaches are hypothesis-driven experiments that select genes based on *a priori* knowledge about the molecular physiology that underlies the development of obesity (3). In contrast, linkage analyses are hypothesis-generating experiments that rely on the relatedness of the study participants and test whether genomic regions are co-segregating with obesity phenotypes across generations (13). Although these molecular approaches have advanced the understanding of monogenic obesity (12), progress identifying genes

implicated in common obesity using candidate gene and linkage analyses has been extremely limited (3).

More recently, the search for common obesity candidate genes has been expedited by genome wide association studies (GWAS). The GWAS approach is a hypothesis-generating experiment that extends the idea of linkage analyses by screening single nucleotide polymorphisms (SNPs) throughout the genome for association with a phenotype of interest (14). In 2007, the first GWAS for BMI and body weight identified a gene that encodes fat mass and obesity-associated protein (*FTO)* (15) and subsequent investigations were quick to expand the list of gene reproducibly associated with obesity (16–18). Current meta-analysis based on all obesity GWAS (249,796 participants and 2.8 million markers) reported that 32 genetic loci were now reproducibly associated with variation in BMI (19). Although GWAS has expedited the discovery of human obesity genes and provided opportunities to define mechanistic insights into the regulation of body weight, the heritable component of obesity explained by the aggregate of these loci remains low (<2%) (19).

1.3 GENE-BY-ENVIRONMENT INTERACTIONS

Increasingly, gene-by-environment interactions have been identified as a potential strategy to partially account for the "missing heritability" that is attributed to complex phenotypes like obesity (20). Gene-by-environment interactions (GEI) studies are aimed at determining whether the response or adaptation to an environmental exposure is conditional upon the genotype of the individual (21). Observational studies can test for GEIs when information on both environmental exposure (lifestyle and dietary intake) and genetic factors (SNPs) are collected in large cohort studies (22).

1.4 POLYUNSATURATED FATTY ACIDS AND HEALTH

Polyunsaturated fatty acids (PUFAs) are essential fats that are required by humans and other mammals for survival (23). Two types of naturally occurring PUFAs are found in mammalian tissues that include the n-6 series (n-6 PUFAs) derived from *cis*-linoleic

acid (LA) and the n-3 series (n-3 PUFAs) derived from α-linolenic acid (ALA) (24). Dietary sources of n-6 PUFAs include cereals, eggs, poultry, most vegetable oils, whole-grain breads, baked goods, margarine as well as sunflower oil, safflower oil, and corn oils (25). Dietary sources of n-3 PUFAs include canola oil, flaxseed oil, linseed oil, rapeseed oil, walnuts, leafy green vegetables as well as marine foods (25). Animal and human studies demonstrate that dietary intake of PUFAs impact diverse physiological processes including immune regulation (26), inflammation (24), neurological conditions (26), cardiovascular disease (26), cancer (27), diabetes (26) as well as metabolic syndrome (23). Moreover, accumulating evidence suggest that regular consumption of marine derived n-3 polyunsaturated fatty acids (n-3 PUFAs), namely eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), may also have anti-obesity effects (28).

1.5 ANTI-OBESITY EFFECTS of n-3 PUFA INTAKE IN ANIMALS

In rodent models, significant reductions in fat mass were observed when dietary n-3 PUFAs were substituted for saturated fats (29), monosaturated fats (30), and n-6 polyunsaturated fats (31). Supplementing mice on a high fat diet with n-3 PUFAs was also associated with less weight gain (32, 33) and minimized visceral fat accumulation (34, 35). Although a growing body of literature demonstrates that n-3 PUFA intake can reduce body fat in animal studies, the anti-obesity effects of n-3 PUFAs in humans has not been fully characterized.

1.6 ANTI-OBESITY EFFECTS OF n-3 PUFA INTAKE IN HUMANS

A limited number of studies have evaluated the extent to which n-3 PUFA intake influences adiposity in humans. In healthy adults, replacing control diets with n-3 PUFAs (1.1 g/day EPA and 0.7 g/day DHA) for 3 weeks, was associated with increased fatty acid oxidation (22%) at rest and reductions in body fat (-0.9 kg) (36). Similarly, supplementing n-3 PUFAs (1.08 g/day EPA and 0.7 g/day DHA) for 3 months in obese women with T2D, was associated with reduced total fat mass (3.5%) and smaller adipocyte diameter (6%) (37). In young adults on an energy-restricted diet (8 weeks), the

addition of either lean fish (0.3 g/day n-3 PUFA), fatty fish (3.0 g/day n-3 PUFA), or fish oil (1.5 g/day n-3 PUFA), was associated with greater weight loss (~1kg) relative to similar diets without seafood or fish oil supplements (38). Taken together, these studies demonstrate that modest increases in n-3 PUFA intake (0.3-3.0 g/day) over short periods of time (< 3 months) may be effective in reducing body fat in humans.

Observational studies have provided indirect evidence that long-term n-3 PUFA intake may reduce obesity. Baseline data from the Health Professionals Follow-Up (HPFU) study, a 12 year prospective study investigating stroke in 43,671 men, found that regular consumption of fish enriched with n-3 PUFAs (>5 times a week) was inversely associated with proportion of men that were overweight (39). In contrast, the Nurse's Health (NH) Study, a 14 year prospective study that investigated the effects of fish consumption on stroke in 79,839 women, found that higher intakes (>5 times a week) of fish containing n-3 PUFAs was positively associated with BMI, independent of energy intake (40). Although these studies provide mixed results regarding the contribution of long-term n-3 PUFA intake to changes in obesity, it is not clear whether these differences are related to gender-specific effects of n-3 PUFA intake on obesity or dietary assessment tools that were not sensitive enough to precisely quantify n-3 PUFA intake (28). Methods that precisely track consumption of n-3 PUFA intake, such as a physiological biomarker, may help clarify the extent to which n-3 PUFA intake influences the risk of developing obesity.

In summary, rodent studies demonstrate that supplementing the diet with n-3 PUFAs attenuated weight gain and reduces the accumulation of body fat. Consistent with these findings, human studies indicate that modest consumption of n-3 PUFA intake (0.3-3.0 g/day) can reduce body weight in overweight or obese individuals (36–38). Although animal and human studies suggest modest levels of n-3 PUFA intake may have anti-obesity effects (28), the extent to which intake of n-3 PUFA modifies the genetic risk of developing obesity is unknown.

1.7 MEASURING n-3 PUFA INTAKE

Evidence that consumption of n-3 PUFAs modifies genetic risk of developing obesity in large cohort studies is limited, in part because estimates of dietary EPA and DHA intakes are difficult to quantify (41). Estimates of dietary intake are traditionally captured by either self-reported food frequency questionnaires (FFQs) or 24-hour food recall interviews (24HRs) (42). FFQs ask participants to select from a number of food items and report the frequency of consumption and portion size during a specific time interval (week, month, or year) (43). In contrast, 24HRs ask participants to quantify all liquids and foods consumed in the past 24 hour period (44); however it is widely recognized that a single 24HR is not representative of "usual intake" (45). Estimating n-3 PUFA intake in large cohort studies using self-report questionnaires has proven difficult, in part, because methods which are feasible to collect in large populations (i.e., FFQ) suffer large error and biases (46, 47), whereas more reliable methods (i.e., multiple 24HR) can be prohibitively time consuming and costly (48, 49).

Alternative dietary assessment approaches such as biomarkers, may provide a more accurate measure of n-3 PUFA intake in large epidemiologic studies (50). Membrane composition of red blood cell (RBC) are effective biomarkers of n-3 PUFA intake (51), however their application in large cohort studies is limited because measurements are technically challenging, expensive, and time consuming (41). Nitrogen stable isotope ratios ($\delta^{15}N$) have been developed as a validated biomarker of n-3 PUFA intake in large samples (41, 52). In a cohort of Yup'ik adults (n=496), $\delta^{15}N$ of RBC was positively correlated with EPA (r=0.84) and DHA (r=0.75) in RBC (41). Given that measurement of $\delta^{15}N$ in RBC is inexpensive, accurate, and can easily be assayed in large numbers of samples (41), estimating n-3 PUFA intake using $\delta^{15}N$ in RBC as a validated biomarker for EPA and DHA consumption in Yup'ik people may increase the ability to detect gene-by-environment interactions.

1.8 CENTER FOR ALASKA NATIVE HEALTH RESEARCH STUDY

Southwest Alaska is home to approximately 20,000 Yup'ik people who reside in approximately 50 rural villages (53). The Center for Alaska Native Health Research (CANHR) study is a long-term epidemiological investigation based in Southwest Alaska that is dedicated to understanding genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik people (54). For the past 10 years, the CANHR field team has worked with 10 remote villages (coastal/inland). The rural communities CANHR works with are not on the road system and most of the residents engage in subsistence hunting and fishing to generate much of their food. Most residents speak the indigenous Yup'ik language and the culture of this region is considered one of the most intact in the state. CANHR investigators have collected health histories, family history information, body measurements, dietary assessments and fasting blood samples (54). The prevalence of obesity among CANHR participants is comparable to the general US population, yet the prevalence of metabolic syndrome (8.6 % in Yup'ik men and 19.8% in Yup'ik women) (55) and T2D (<3% in Yup'ik people) (54) is significantly less than that observed in the general US population (56, 57). Based on these observations, the health of Yup'ik people, may in part, be influenced by their subsistence diet, including regular consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) (58, 59). Although the mechanisms that allow Yup'ik people to carry excess body fat without developing features of metabolic syndrome and T2D are unknown, dietary intake of n-3 PUFAs and genetic factors (60) are likely to be relevant.

1.9 SCOPE OF DISSERTATION

Given that gene-by-environment interactions are hypothesized to account for the missing heritability attributed to obesity (20), cross-sectional studies in a Yup'ik study population that demonstrate n-3 PUFA intake modifies genetic factors associated with obesity may help elucidate important mechanisms that can modulate the risk of obesity through dietary interventions. Regular consumption of n-3 PUFAs has been associated

with protection from obesity-related diseases in Yupik people (61, 62), yet information regarding whether the anti-obesity effects attributed to n-3 PUFA are dependent upon a specific genotype remains limited. This dissertation examined the contribution of interactions between n-3 PUFA intake and obesity candidate genes in a Yup'ik study population with up to 30-fold variation in n-3 PUFA intake. We selected the candidate genes based on a strategy that identified obesity candidate genes with 1) strong physiological evidence; 2) candidate genes implicated in obesity whole-genome linkage studies and known to be regulated by PUFAs; and 3) candidate genes reproducibly implicated in obesity genome-wide association studies.

1.10 CANDIDATE GENES

1.10.1 PHYSIOLOGY

We selected carnitine palmitoyltransferase 1A (*CPT1A*) as a candidate gene because this gene has a strong physiological role in regulating fatty acid oxidation in the liver and unique polymorphisms that are common among Eskimo/ Inuit populations (63). Impaired fatty acid oxidation is associated with obesity (64, 65) and may have consequences that include hepatic steatosis, hepatic insulin resistance, and impaired hepatic lipid handeling (66). Carnitine palmitoyltransferase 1 (CPT1) is a gene that controls mitochondrial fatty acid oxidation in skeletal, adipose, and liver tissue (67). Mammalian tissues express three *CPT1* isoforms: *CPT1A* (liver), *CPT1B* (muscle) and *CPT1C* (brain), which are encoded on separate genes (68–70). Functional studies indicate that n-3 PUFA increase mitochondrial fatty acid oxidation, largely by stimulating the activity of *CPT1* (71). A meta-analysis for obesity whole-genome linkage studies implicates the 11q region that harbors *CPT1A* as an obesity linked gene region harboring potential candidate genes (72).

1.10.2 WHOLE-GENOME LINKAGE STUDIES

We selected candidate genes for this part of our study from chromosomal regions that have been identified in three or more linkage studies and functional studies showing

these genes are regulated by n-3 PUFAs. These three candidate genes included: stearoyl CoA desaturase (*SCD*), insulin sensitive glucose transporter (*SLC2A4*), and steroyl regulatory element binding protein (*SREBF1).*

SCD is the rate-controlling enzyme catalyzing the biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acid substrates (73). SCD1 *(*mouse *SCD* homolog*)* deficiency in mice is associated with increased energy expenditure (74), reduced adiposity (75), increased insulin sensitivity (76), protection against hypertriglyceridemia (77), and elevated HDL levels (77). Consumption of n-3 PUFA was associated with inhibition of *SCD1* transcription in the liver (78) and adipocytes (79). Whole-genome studies in humans have demonstrated that the 10q region harboring the SCD gene is linked with obesity (80–82).

SLC2A4 codes for the glucose transporter (GLUT4) that is a specialized insulin-stimulated glucose transporter that regulates glucose uptake in skeletal muscle, heart, and adipose tissue (83). In the presence of insulin, GLUT4 is translocated to the cell membrane and facilitates glucose disposal (84). Inhibition of GLUT4 transport has been implicated in insulin resistance (85). Rodent studies show that n-3 PUFA intake increase GLUT4 transport (86) and GLUT4 protein levels (87, 88). Finally, whole-genome linkage studies have demonstrated the 17p region that harbors *SLC2A4* is linked with obesity-related phenotypes that include plasma leptin (89), BMI (90) and plasma adiponectin levels (91).

SREBF1 is a transcription factor that plays a critical role in energy homeostasis by promoting glycolysis, lipogenesis, and adipogenesis (92). PUFAs influence the activity of SREBF1 by increasing *SREBF1* mRNA turnover (93, 94). Whole-genome linkage studies have demonstrated the 17p11 region harboring the *SREBF1* gene is linked to obesity-related traits that include plasma leptin (89), BMI (90), and type 2 diabetes (95).

1.10.3 GENOME-WIDE ASSOCIATION STUDIES

We selected candidate genes for this part of our studies based on highly replicated SNPs that were identified in large-scale obesity GWAS. These candidate genes included:

FTO, ETV5, NEGR1, TMEM18, GNPDA2, BDNF, MTCH2, SH2B1, MC4R and *KCD15.*

In 2007, the first GWAS for T2D identified a SNP that was associated with BMI in the first intron of a gene called fat mass and obesity-associated protein (*FTO*) (15). *FTO* is a gene encoding a 2-oxoglutarate-dependent nucleic acid demethylase that is responsible for catalyzing the demethylation of 3-methylthymine in single-stranded DNA (96, 97). To date this is the most highly replicated obesity candidate gene (98). Candidate gene studies in European (99), African (100), and Asian (101) study populations have largely confirmed the association of *FTO* with BMI.

ETV5 is a transcription factor (ets variation 5) that is involved in a diverse range of developmental and pathogenic processes (102). In 2007, large-scale GWAS for obesity reported a SNP near ETV5 (rs7647305) that was associated with variation in BMI (17). Functional studies demonstrate that *ETV5* knockout mice had reduced body weight compared to controls (103).

NEGR1 (neuronal growth regulator 1) is a member of the immunoglobulin superfamily of cell adhesion molecules (104) that is highly expressed in the brain (hypothalamus) and adipose tissue (105). In 2009, large-scale GWAS for obesity reported a SNP in *NEGR1* (rs2815752) that was associated with variation in BMI (18). In mice, loss of NEGR1 function in mice was associated with reduced body mass, relative to controls (106).

TMEM18 (transmembrane protein 18) is transmembrane protein that expressed throughout the brain and involved with neural cell migration (107). In 2007, large-scale GWAS for obesity reported a SNP in *TMEM18* (rs7561317) that was associated with variation in BMI (17). In rats, body weight was positively associated with expression of *TMEM18* in the prefrontal cortex of the brain (108).

GNPDA2 (glucosamine-6-phosphate deaminase) is an enzyme that catalyzes the conversion of glucosamine-6-phosphate to fructose-6-phosphate (109). In 2009, large-scale GWAS for obesity reported a SNP in *GNPDA2* (rs10938397) that was associated with variation in BMI (18). Functional studies have yet to define the role of *GNPDA2* in regulation of body weight.

BDNF (brain-derived neurotrophic factor) is a member of the neurotrophic family of growth factors that is expresses throughout the central nervous system (110). In 2009, large-scale GWAS for obesity reported a SNP in *BDNF* (rs6265) that was associated with variation in BMI (18). Functional studies show that *BDNF* activity is associated with changes in eating behavior (111).

MTCH2 (mitochondrial carrier homolog 2) is a member of the mitochondrial carrier proteins that has been implicated in regulation of cell growth, motility, and tumorigenicity (112). In 2009, large-scale GWAS for obesity reported a SNP in *MTCH2* (rs10838738) that was associated with variation in BMI (18). Functional studies have yet to define the role of *MTCH2* in the development of obesity.

SH2B1 (SH2B adaptor protein) is an adapter protein for tyrosine kinase receptors that is highly expressed in skeletal muscles and ovaries (113). Several large-scale GWAS for obesity reported a SNP in *SH2B1* (rs7498665) that was associated with variation in BMI (17, 18). In mice, SH2B1 is required for normal body weight maintenance and acts as an endogenous enhancer of leptin sensitivity (114).

MC4R (melanocortin 4 receptor) belongs to the melanocortin family of receptors that have been implicated in a wide range of physiological functions including energy homeostasis (115). In 2008, a large-scale GWAS reported a SNP in MC4R (rs1778213) was associated with BMI (16). Functional studies demonstrate that MC4R knockout mice are overweight (116).

KCD15 (potassium channel tetramerization domain containing 15) is a member of the volt-gated potassium channel proteins that maintain cellular membrane potential (117). In 2007, large-scale GWAS for obesity reported a SNP in KCD15 (rs29941) that was associated with variation in BMI (17). Functional studies have yet to define the role of *KCD15* in the development of obesity.

1.10.3.1 CANDIDATE GENE SUMMARY

Numerous candidate genes have been implicated in obesity pathophysiology, however there is currently a lack of information that characterizes the interplay between

genetic factors and consumption of n-3 PUFA that may facilitate the choice of more effective and specific measures of obesity prevention based upon individualized genetic make-up.

1.11 DISSERTATION SPECIFIC AIMS

1) Determine whether single nucleotide polymorphism (SNPs) in obesity candidate genes were associated with obesity phenotypes in a Yup'ik study population.
2) Identify SNPs that interact with n-3 PUFA intake and modify the genetic associations with obesity phenotypes in a population with highly variable intake of n-3 PUFAs.

1.12 APPROACH

This dissertation will address the specific aims outlined above using CANHR data collected between 2003 and 2008. SNP selection was based on criteria that included: (1) maximally informative tagging SNPs within or near each obesity candidate genes using publicly available HapMap data, (2) previously identified SNPs that were associated with obesity-related phenotypes, and (3) SNPs identified by resequencing 30 unrelated Yup'ik participants enrolled in the CANHR study. Genetic associations and gene-diet interaction analyses were conducted using the program ASSOC (118) in the Statistical Analysis for Genetic Epidemiology (S.A.G.E. 2009) software package that can incorporate complex pedigree data, covariates and interactions into association analysis. Significance of association was evaluated at conventional significance levels ($p \leq 0.05$) and multiple test correction was adjusted according to the number of non-redundant genetic markers.

1.13 DISSERTATION ORGANIZATION

In Chapter 2, I present a published manuscript where we examined a gene that controls hepatic lipid oxidation called *carnitine* palmitoyltransferase 1A (*CPT1A*). Chapter 3 presents a manuscript that examines genes regulated by n-3 PUFA intake that were implicated in multiple obesity whole-genome linkage studies. Chapter 4

investigates candidate genes identified in multiple obesity genome-wide association studies (GWAS) using a genetic risk score. Chapter 5 presents the discussion and future direction.

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GENETIC POLYMORPHISMS IN *CARNITINE PALMITOYLTRANSFERASE 1A* GENE ARE ASSOCIATED WITH VARIATION IN BODY COMPOSITION AND FASTING LIPID TRAITS IN YUP'IK PEOPLE[1]

2.1 ABSTRACT

Variants of carnitine palmitoyltransferase 1A (*CPT1A*), a key hepatic lipid oxidation enzyme, may influence how fatty acid oxidation contributes to obesity and metabolic outcomes. *CPT1A* is regulated by diet, suggesting interactions between gene variants and diet may influence outcomes. The objective of this study was to test the association of *CPT1A* variants with body composition and lipids, mediated by consumption of polyunsaturated fatty acids (PUFAs). Obesity phenotypes and fasting lipids were measured in a cross-sectional sample of Yup'ik individuals (n=1141) from the Center of Alaska Native Health Research (CANHR) study. Twenty-eight tagging *CPT1A* SNPs were evaluated with outcomes of interest in regression models accounting for family structure. Several *CPT1A* polymorphisms were associated with HDL-cholesterol and obesity phenotypes. The P479L (rs80356779) variant was associated with all obesity-related traits and fasting HDL-cholesterol. Interestingly, the association of P479L with HDL-cholesterol was still significant after correcting for either BMI, percent body fat (PBF), and waist circumference (WC). Our findings are consistent with the hypothesis that the L479 allele of the *CPT1A* P479L variant confers a selective advantage that is both cardio-protective (through increased HDL-cholesterol) and associated with reduced adiposity.

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[1] Lemas, DJ, Weiner HW, O'Brien DM, Hopkins S, Stanhope KL, Havel PJ, *et al.* 2012. Genetic Polymorphisms in *Carnitine Palmitoyltransferase 1A* Gene are Associated with Variation in Body Composition and Fasting Lipid Traits in Yup'ik People. Journal of Lipid Research. January 1;53(1):175-84.

2.2 INTRODUCTION

Obesity is associated with a series of metabolic conditions clinically referred to as metabolic syndrome, which includes hypertension, dyslipidemia, hyperglycemia, and the development of type 2 diabetes (T2D). Approximately sixty percent of obese individuals have metabolic complications (1), however "healthy obese" individuals have been identified with excessive accumulation of body fat that does not translate to dyslipidemia and insulin resistance (2, 3). For example, some Eskimo/Inuit people indigenous to Alaska are obese, but have historically demonstrated low prevalence of insulin resistance, metabolic syndrome, and T2D (4–7). Specifically, Yup'ik people living in Southwest Alaska have obesity prevalence comparable to the general US population, yet the prevalence of metabolic syndrome (8) and T2D (9) is significantly less than that observed in the general US population (10, 11). Although the mechanisms that allow Yup'ik people to carry excess body fat without developing features of metabolic syndrome and T2D are unknown, dietary and genetic factors are likely to be relevant (12, 13). Because weight loss as a treatment for obesity related co-morbidities is difficult to achieve and maintain (14–17), understanding the underlying mechanisms that protect this population from features of metabolic syndrome despite their adiposity would have implications for treatment of obesity without the necessity of weight loss.

It has been proposed that the 'healthy obesity' observed in Yup'ik individuals is in part related to exposure to a diet rich on n-3 polyunsaturated fatty acids (n-3 PUFAs) (18, 19). n-3 PUFAs consumed by Yup'ik people are principally composed of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), and PUFA intake is 20 times greater than the current mean intake of the general US population (4.1 ± 0.5 g/day versus 0.05 g/day in men and 2.8 ± 0.3 g/day versus 0.09 g/day in women) (20, 21). Cross-sectional studies in a Yup'ik population offer a unique opportunity to examine the association of elevated n-3 PUFA exposure with body composition, fasting lipids and lipoprotein levels. Studies in both animals and humans have demonstrated that EPA and DHA impact body composition and circulating fasting lipid levels by modulating gene expression to favor increased fatty acid oxidation and reduction of fat deposition (22).

Evidence that elevated n-3 PUFA consumption has a direct influence on 'healthy' obesity remains inconclusive (12, 13) and warrants experimental designs which evaluate gene-diet interactions that may mediate this effect in populations with elevated daily dietary intake of n-3 PUFA.

Mitochondrial carnitine palmitoyltransferase 1 (*CPT1*), a member of the carnitine palmitoyltransferase family, is a gene that controls fatty acid oxidation in skeletal, adipose, and liver tissue (23). Fatty acid oxidation is often impaired in the obese condition (24, 25), which may contribute to hepatic steatosis, hepatic insulin resistance, and impaired hepatic lipid handeling (26). CPT1 as a major control point for fatty acid oxidation may therefore be a key player in "healthy obesity", especially if certain single nucleotide polymorphisms (SNPs) are resistant to impaired fatty acid oxidation which often accompanies obesity. Interestingly, n-3 PUFA increases mitochondrial fatty acid oxidation by stimulating the activity of *CPT1* (27), the interaction between n-3 PUFAs and SNPs in CPT1 may improve lipid profiles.

Mammalian tissues express three *CPT1* isoforms: *CPT1A* (liver), *CPT1B* (muscle) and *CPT1C* (brain), which are encoded on separate genes (28–30). In the presence of L-carnitine, *CPT1* facilitates the transfer of long-chain fatty acids (LCFA) across the mitochondrial membrane for β-oxidation (31). Mitochondrial β-oxidation of dietary and endogenous LCFA is tightly regulated through allosteric inhibition of *CPT1* by malonyl-CoA, an intermediate in fatty acid synthesis (32). In liver cells, the partnership between malonyl-CoA and CPT1A has been shown to be a key regulatory point that modulates the oxidation of dietary and endogenous LCFA (33). Although, *CPT1A* is a candidate gene for obesity (34) and *CPT1A* SNPs are associated with elevated fasting HDL-cholesterol levels (35); it is unknown whether the interactions between n-3 PUFA intake and *CPT1A* SNPs influence changes in body composition and fasting lipids.

In this study we tested the hypothesis that SNPs within or near the *CPT1A* gene are associated with body composition and fasting lipid phenotypes in a large cross sectional cohort of Yup'ik people, a population whose daily dietary intake involves 30-

fold range of exposure of n-3 PUFA, and examined whether these associations were modified by n-3 PUFA intake.

2.3 PARTICIPANTS & METHODS

2.3.1 PARTICIPANTS AND STUDY DESIGN

The Center for Alaska Native Health Research (CANHR) studies genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik people (9). A community-based participatory research framework guides all CANHR investigations, such that participant ascertainment is open to all members of the community meeting a specified age minimum. Recruitment of Yup'ik participants was initiated in 2003 and continues in 11 Southwest Alaska communities. All residents 14 years of age and older are invited to participate and the resulting distribution of age in our study sample reflects the age distribution among eligible participants according to 2000 U.S. census data. Participants sign informed-consent documents before entering the study using protocols that were approved by the University of Alaska Institutional Review Board, the National and Alaska Area Indian Health Service Institutional Review Boards, and the Yukon-Kuskokwim Health Corporation Human Studies Committee. The analyses in this report were performed on 1141 non-pregnant Yup'ik participants with ages that ranged between 14 and 94 years at the time of enrollment.

2.3.2 ANTHROPOMETERIC AND BIOCHEMICAL MEASUREMENTS

Anthropometric measurements were obtained by trained staff using protocols from the NHANES III Anthropometric Procedures Manual (36) as previously described (8). These measurements included height, weight and 4 circumferences (waist, hip, triceps, and thigh). Percent body fat (PBF) was measured by electrical bioimpedance using a Tanita TBF-300A body composition analyzer (Tanita Corp, Arlington Heights, IL, U.S.A.). Blood samples were collected from participants after an overnight fast, and lipoprotein measures including total cholesterol, HDL-cholesterol, LDL-cholesterol,

VLDL-cholesterol, apolipoprotein A1 and plasma triglycerides levels were assayed as previously described in Boyer *et al.* (8).

2.3.3 BIOMARKER FOR MARINE n-3 PUFA INTAKE

n-3 PUFA intake was assessed in Yup'ik individuals using the nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells (RBC) as previously described (37). RBC aliquots were autoclaved for 20 minutes at 121° C to destroy blood-borne pathogens, and samples were weighed into 3.5 x 3.75 mm tin capsules and freeze dried to a final mass of 0.2 - 0.4 mg. Samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio mass spectrometry, using a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo-Finnigan Inc., Breman, Germany). Isotope ratios are analyzed relative to IAEA-certified reference materials calibrated to atmospheric nitrogen, for which $^{15}N/^{14}N = 0.0036765$. By convention and for ease of interpretation, isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \cdot 1000‰$. We concurrently prepared and ran multiple laboratory standards (peptone, $\delta^{15}N = 7.00$) to assess analytical accuracy and precision; these were analyzed after every eighth sample and gave values of $\delta^{15}N = 7.01 \pm 0.24‰$ (mean ± SD). The range of isotopic variation in our dataset (9‰) was very large relative to analytical precision (0.2‰). We modeled the effects of n-3 PUFA intake as a categorical variable, which is hereafter referred to as $\delta^{15}N$.

2.3.4 SNP SELECTION AND GENOTYPING

A comprehensive list of DNA variants were selected for genotyping within and near (5 kb upstream and 5 kb downstream) the *CPT1A* gene collected from HapMap data, release #3, National Center for Biotechnology Information (NCBI) B36, dbSNP 126 (38). Given that no publically available genotypic information exists on Yup'ik people, we referenced the Caucasian (CEU) and Han Chinese (CHB) populations in HapMap using

the SeattleSNPs database (http://pga.mbt.washington.edu/) to identify potential genetic variants that may be common in our study population. A set of 27 maximally informative tagging SNPs (tSNPs) were selected to represent common linkage disequilibrium clusters with the LDselect algorithm as implemented in the MultiPop-TagSelect program using thresholds of r^2=0.80 and minor allele frequency >1% (39, 40). We chose to relax our MAF criteria to include SNPs with MAF >0.01 in order to genotype tagging SNPs in the *CPT1A* gene which may be common (MAF≥0.05) in Yup'ik people despite being rare (MAF<0.05) in CEU and CHB populations. We also included the non-synonymous P479L (rs80356779) *CPT1A* SNP for genotyping based on previous associations with elevated plasma HDL-cholesterol and apolipoprotein A1 levels in the Greenland Inuit (35). Genotyping of the 28 SNPs including P479L was carried out by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight spectrometry on a Sequenom iPLEX platform at the Broad Institute (41). Linkage disequilibrium (LD) among SNPs was based on pairwise haplotype frequencies calculated using the hapfreq command in the FBAT program (42).

2.3.5 QUALITY CONTROL OF PHENOTYPIC AND GENOTYPIC DATA

Simple linear models were fit to each of the outcome variables using all of the covariates (age, sex, community membership) included in the association models, and the distributions of the residuals were examined for normality with the R statistical programming language (v2.10.1, R Development Core 2009). Box-Cox transformations were applied to traits whose residuals did not follow a normal distribution (43). Family data was extracted from a Progeny database (Progeny Software LLC, South Bend, IN, U.S.A.) and merged into a single extended pedigree using PedMerge (44). Genotypic data were tested for Mendelian inconsistencies using PEDCHECK (45). In this sample, Illumina IV linkage panel (Illumina, Inc., San Diego, CA, USA) genotypes were available from ongoing linkage study and were used to construct principal components of ancestry (PCA) using the PCA program in the EIGENSTRAT analysis package (46).

The second PCA discriminated the individuals in the study into two groups that correspond to the proximity of the community to the coast. Based on this observation, we defined a dichotomous community group variable. We assessed Hardy-Weinberg equilibrium (HWE) using PLINK (v1.07) (47) and determined allele frequencies for each SNP using the FREQ module in the program Statistical Analysis for Genetic Epidemiology (S.A.G.E 2009). The present study restricted analysis to only include SNPs with MAF $\geq$ 5% that did not deviate from HWE after Bonferroni correction ($p<0.002$).

2.3.6 ASSOCIATION ANALYSIS

Each SNP was tested for association with obesity-related phenotypes using the program ASSOC (48) in the S.A.G.E software package that can incorporate complex pedigree data, covariates and interactions into association analysis. We included both demographic (age, community, and sex) and environmental variables ($\delta^{15}N$) in the ASSOC analysis. Likelihood ratio statistics were calculated to compare 3 nested models and test the null hypothesis of no association between *CPT1A* SNPs and obesity traits after including demographic and environmental covariates. Effect sizes (β) were presented as the change in transformed phenotypes according to minor allele that was determined in a linear model adjusted for demographic and environmental covariates.

Model 1 included baseline covariates (age, sex, community membership, and $\delta^{15}N$ quartiles); Model 2 included baseline covariates and SNP to test for an additive genetic effect of SNP (defined as the number of minor alleles); and Model 3 included baseline covariates, the additive genetic effect of SNP, and interactions between the additive genetic effect and $\delta^{15}N$ quartiles. Note that the model 3 is the only model to test directly gene-diet interaction under the null hypothesis. We have treated each phenotype being tested as representing a separate family of null hypotheses and correct for the number of tests within each family (49). Multiple test correction to control the familywise error rate was calculated according to the number of non-redundant SNPs with MAF $\geq$0.05 that were tested for association and interaction. Given the correlation among neighboring

genetic markers, the effective numbers of non-redundant SNPs in this study was estimated using spectral decomposition of LD matrices (50, 51).

2.4 RESULTS

2.4.1 CHARACTERISTICS OF YUP'IK PARTICIPANTS

General clinical characteristics and descriptive statistics on Yup'ik men and women are presented in **Table 2.7.1.** Yup'ik women in this study had a mean age of 37.6 (± 17.3) years and men reported a mean age of 35.9 (±17.3) years. Women had significantly greater BMI, percentage body fat, hip circumference, fasting total cholesterol, HDL-cholesterol, and ApoA1 levels compared to men ($p<0.05$). According to the standard cut-off points for overweight (BMI 25-29.9 kg/m^2) and obese (BMI ≥ 30 kg/m^2), 28.6% of women and 30.7% of men were overweight while 37.0% of women and 7.9% of the men were classified as obese.

2.4.2 DISTRIBUTION OF $\delta^{15}N$ IN STUDY POPULATION

In 1138 Yup'ik participants, we assessed n-3 PUFA intake using RBC $\delta^{15}N$ as a biomarker of EPA and DHA intake. Summary statistics grouped by gender and $\delta^{15}N$ quartiles are reported in **Table 2.7.2.** The mean $\delta^{15}N$ value was 9.0‰ with a range of 6.2‰-15.2‰. This range was large relative to analytical precision (0.2‰) and was 3.75 times greater than the RBC (clot) $\delta^{15}N$ values previously reported for a random sample of US residents (52). According to the linear relationship between RBC $\delta^{15}N$ and RBC EPA reported elsewhere for this population (37), the corresponding mean EPA (% RBC fatty acids) was 2.66% with a range of ~0%-9.1%. Measurement of $\delta^{15}N$ by gender yielded means of 9.1‰ for females and 8.8‰ for males. The mean RBC $\delta^{15}N$ values by quartile were: 7.3‰, 8.2‰, 9.1‰, and 11.0‰ in quartiles 1-4 respectively. These values correspond to EPA (% RBC fatty acids) quartile means of: 0.9%, 1.8%, 2.8%, and 4.7% (37). The standard deviation of $\delta^{15}N$ in this sample did not differ by gender (1.5‰ for both females and males).

2.4.3 GENETIC VARIATION IN THE *CPT1A* GENE

DNA was available in 1141 Yup'ik participants and the mean number of individuals successfully genotyped was 1078 (range of 986-1137, depending on the SNP). Twenty-eight *CPT1A* SNPs were genotyped with a mean success rate of 94.7% (range 76.1-99.7%). In this sample, 4 SNPs were monomorphic, 12 SNPs had MAF <0.05 and >0.01, and 12 SNPs had MAF ≥ 0.05. Genotyping results for SNPs with MAF ≥0.05 are presented in **Table 2.7.3**. The rs2924697 SNP (MAF=0.28) was the only polymorphism with MAF ≥0.05 that deviated significantly from Hardy-Weinberg proportions and was excluded from the analysis. The non-synonymous P479L SNP was common in our sample and the major L479 allele had a frequency of 0.74. We selected the 11 *CPT1A* SNPs with MAF ≥ 0.05 that did not deviate from HWE proportions for genetic analysis (**Table 2.7.3**). The spectral decomposition of LD matrix (51) estimated that 8 of the 11 markers with MAF ≥0.05 were non-redundant genetic markers and corrected our analysis for 8 tests, setting the per-test α level to <0.0063 (two-tailed).

2.4.4 *CPT1A* SNPS AND FASTING LIPID PARAMETERS

The results of association analysis between fasting lipid traits and *CPT1A* SNPs with MAF ≥ 0.05 are summarized in **Figure 2.6.1** and **Table 2.7.4**. HDL-cholesterol was significantly associated with 7 SNPs: rs2278908 (p=0.0007, β=-2.3, SE=0.7), rs3019598 (p=0.0014, β=-2.2, SE=0.7), P479L (p=0.0001, β=-1.0, SE=0.3), rs11228372 (p=0.0013, β=-1.2, SE=0.4), rs11228373 (p<0.0001, β=-1.3, SE=0.3), rs3019594 (p<0.0001, β=-1.4, SE=0.3) and rs597316 (p=0.0014, β=-2.2, SE=0.7). The rs11228373 and rs3019594 SNPs were also significantly associated with ApoA1 (p=0.0014, β=-1.1, SE=0.4; and p=0.0008, β=-1.2, SE=0.4, respectively), and total cholesterol (p=0.0063, β=-0.7, SE=0.2; and p=0.0031, β=-0.7, SE=0.2, respectively) (**Table 2.7.4**). Note that rs11228373 and rs3019594 are in moderately strong LD (r^2=0.75). The P479L variant was also associated with HDL-cholesterol (p=0.0001) and was not in strong LD with either the rs11228373 (r^2=0.58) or rs3019594 (r^2=0.61) SNPs (**Supplementary Table**

2.9.1). Our model predicted that individuals homozygous for the common allele (L479) of P479L had elevated fasting HDL-cholesterol levels compared to individuals homozygous for the P479L minor allele (P479) (**Table 2.7.6**). After adjusting Model 2 for BMI, the *CPT1A* SNPs (rs2278908, rs3019598, P479L, rs11228373, rs3019594, and rs597316) associated with fasting total cholesterol, HDL-cholesterol, ApoA1 were still significant (**Supplementary Table 2.9.2**).

2.4.5 *CPT1A* SNPS AND OBESITY PHENOTYPES

The results of association analysis between obesity traits and *CPT1A* SNPs with MAF $\geq$ 0.05 are summarized in **Figure 2.6.2** and **Table 2.7.5**. Thigh circumference was associated with 7 SNPs: rs2278908 (p=0.0024, β=2.1, SE=0.7), rs2278907 (p=0.0002, β=1.9, SE=0.5), P479L (p<0.0001, β=1.2, SE=0.3), rs4930248 (p=0.0042, β=1.3, SE=0.5), rs11228372 (p=0.0011, β=1.3, SE=0.4), rs11228373 (p=0.0006, β=1.1, SE=0.3), and rs3019594 (p=0.0001, β=1.3, SE=0.3). Hip circumference was associated with 5 SNPs: rs2278907 (p=0.0057, β=1.2, SE=0.4), P479L (p<0.0001, β=0.9, SE=0.2), rs11228372 (p=0.0034, β=0.9, SE=0.3), rs11228373 (p=0.0063, β=0.7, SE=0.3) and rs3019594 (p=0.0005, β=0.9, SE=0.3).

The P479L SNP and rs3019594 (r^2=0.61 between these SNPs) were SNPs most significantly associated with both thigh circumference and hip circumference (p<0.0001 and p=0.0034, respectively). The P479L SNP was the only SNP associated with all obesity measures that included BMI (p=0.0021, β=0.7, SE=0.2), percentage body fat (p=0.0007, β=0.0007, SE=0.2), hip circumference (p<0.0001, β=0.9, SE=0.2), thigh circumference (p<0.0001, β=1.2, SE=0.3), and waist circumference (p=0.0006, β=1.0, SE=0.3). Individuals homozygous for the common P479L allele (L479) have a lower percentage body fat, smaller BMI, and reduced thigh, hip, and waist circumferences compared to P479 homozygotes (**Table 2.7.6**).

2.5 DISCUSSION

CPT1A has been implicated as candidate obesity gene in a meta-analysis of whole-genome linkage studies (34); however the contribution of *CPT1A* polymorphisms to variation in the metabolic consequences of obesity and obesity phenotypes remains unclear. Our results demonstrate that *CPT1A* polymorphisms are associated with obesity and fasting lipid phenotypes in this Yup'ik study population and may influence the 'healthy obesity' phenotype. Specifically, the P479L SNP was associated with all measures of body composition (BMI, PBF, HC, ThC and WC) and fasting HDL-cholesterol levels. We found that individuals homozygous for the major L479 allele of the P479L variant had reduced body fat and central adiposity relative to individuals homozygous for the minor P479 allele. These data indicate that individuals carrying both copies of the L479 allelle of the non-synonomous P479L variant in *CPT1A* have reduced adiposity and elevated HDL-cholesterol, even after controlling for BMI. Interestingly, when we investigated whether the P479L association with HDL was mediated by other obesity phenotypes, we found the L479 allele was still significantly associatied with HDL-cholesterol after correction for either PBF and WC (**Supplementary Table 2.9.3**). We hypothesize that the L479 allele may contribute to 'healthy obesity' observed in Yup'ik people by modulating hepatic lipid oxidation.

Three studies have previously investigated the influence of *CPT1A* polymorphisms on obesity and lipid phenotypes in humans (35, 53, 54). Hirota and colleagues (53) found no association between *CPT1A* polymorphisms and obesity or fasting lipid phenotypes in Japanese individuals with T2D. In a cross-sectional cohort of French-Canadians, Robitaille *et al.* reported an association between the non-synonymous A275T (rs17610395) SNP with BMI ($p=0.05$) and waist circumference ($p=0.008$) only after accounting for dietary fat intake (54). Finally, in Greenland Inuit, Rajakumar *et al.* showed the L479 allele in the non-synonymous P479L variant was associated with elevated fasting HDL-cholesterol and ApoA1 levels (35).

The present study found an association between SNPs (rs2278908, rs3019598 and rs597316) investigated by Hirota *et al.* with fasting HDL-cholesterol and replicated the

P479L association with fasting HDL-cholesterol and ApoA1 levels reported by Rajakumar *et al.* We have shown that these SNPS were still significantly associated with HDL-cholesterol and ApoA1 after controlling for either BMI, PBF, or WC. Furthermore we used a log likelihood ratio test to determine whether the P479L SNP association with HDL-cholesterol and ApoA1 was independent of the rs11228373 and rs3019594 SNPs. We found that both rs11228373 and rs3019594 SNPs were still significant predictor for HDL-cholesterol and ApoA1 levels even when P479L was already in the model. Given that the rs11228373 and rs3019594 SNPs were in moderately strong linkage disequilibrium with P479L as measured by r^2 (r^2=0.58 and 0.61, respectively), we cannot rule out the possibility that the apparent association with P479L is not due to a true association with either rs11228373 or rs3019594 or both.

We extend the findings of Rajakumar *et al.* to show the L479 allele of P479L is also associated with reduced adiposity in an independent Eskimo/Inuit population. Interestingly, our analysis did not replicate the A275T (rs17610395) association with BMI and WC reported by Robitaille *et al.* because this SNP was not included in the analysis due to a low MAF (MAF=0.02). Factors which may account for differences in results reported in the present study may include, but are not limited to, differences in statistical analysis, small sample size and population stratification (55). Our study, however, benefited from a sample size large enough to detect significant SNP associations and we used a statistical approach that accounts for family structure while allowing for covariates.

Our Yup'ik study population was ideally suited to investigate the contribution of n-3 PUFA and genetic factors to 'healthy obesity' due to the 30-fold range of EPA and DHA consumption (20) which can be precisely estimated in large samples using nitrogen stable isotope ratios from red blood cell samples (37). When we examined whether the interaction between n-3 PUFA intake and *CPT1A* SNPs modifies the association with 'healthy obesity' phenotypes (**Supplementary Table 2.9.4**), we did not find significant gene-diet interactions that modified the association with body composition. Interestingly, interactions between n-3 PUFA intake and rs3794020 and rs2305508 were associated

with HDL-cholesterol and ApoA1 levels, whereby n-3 PUFA intake enhanced the positive association of rs3794020 and rs2305508 minor alleles on these traits. Although n-3 PUFA interactions with genetic factors have received considerable attention, our results should be interpreted with caution given the sample size and nominal significance.

We hypothesize that the observed association between the P479L variant on body composition and fasting lipid phenotypes in the presence of n-3 PUFA intake, may in part explain the presence of a 'healthy obese' phenotype among Yup'ik people. In humans, low rates of endogenous lipid oxidation are associated with obesity (25) and mechanisms that alter an individual's metabolic profile in favor of fatty acid oxidation have been suggested to reduce the accumulation of body fat (56). Consumption of n-3 PUFA increases hepatic fatty acid β-oxidation, primarily through activity of CPT1A (57, 58). Functional studies in fibroblast cells have demonstrated that the L479 allele of P479L variant in *CPT1A* results in a CPT1A enzyme with diminished catalytic activity compared to control cells (59). However, expression of the L479 allele in fibroblasts was also shown to abolish the ability of malonyl-CoA to inhibit CPT1A (59). Interestingly, these data are consistent with a study in rats demonstrating that malonyl-CoA-insensitive CPT1A was more effective than overexpression of wild-type CPT1A at oxidizing lipid substrates (33). Therefore, in the presence of n-3 PUFAs, there may be a net increase in the basal activity of CPT1A among individuals carrying the L479 allele and fatty acids normally packaged in the liver as VLDL will instead be oxidized in the hepatocyte (60). Taken together, we hypothesize that the combined effects of n-3 PUFA intake and the high frequency of the P479L variant in Eskimo/ Inuit populations may influence 'healthy obesity' phenotypes primarily through reduced hepatic VLDL formation and subsequent reductions of plasma triglycerides and VLDL. This model is consistent with our observations that obese Yup'ik people with high intake of n-3 PUFAs have low triglyceride levels, reduced c-reactive protein levels (13), and high circulating HDL-cholesterol levels (12), suggesting that n-3 PUFAs may protect from chronic disease in the presence of obesity.

CPT1A deficiency has been associated with risk for hypoketotic hypoglycemia, hepatic encephalopathy, and sudden infant death syndrome (61–64), as well as muscle cramps, vomiting, and occasional loss of consciousness (59, 65). Nevertheless, the high frequency of the L479 allele in Inuit and Yup'ik people suggested to us, and several others, that it may confer a selective advantage (60, 65, 66). We hypothesized that genetic variants in *CPT1A* may be associated with obesity because of the central role of the CPT1A enzyme in fatty acid oxidation. Our results and those of Rajakumar and colleagues(35) are consistent with a cardio-protective role of the L479 allele of P479L through its association with elevated HDL-cholesterol levels. In this study, we have also shown that genetic variants of *CPT1A* are associated with reduced adiposity and we have replicated the association of elevated fasting HDL-cholesterol and ApoA1 levels with carriers of the L479 allele in this Yup'ik study population. Furthermore, we found that *CPT1A* SNPs associated with HDL-cholesterol and ApoA1 levels were independent of obesity as measured by BMI, PBF, and WC. The P479L variant was not in strong LD (r^2>0.8) with any other *CPT1A* polymorphisms associated with body composition and fasting lipid parameters and suggests that the P479L may have a causal role in 'healthy obesity.' Although we cannot exclude the possibility that other variants are in strong LD with the P479L, our data suggest that the P479L variant in *CPT1A* increases hepatic fatty acid oxidation and may contribute to 'healthy obesity' observed in this Yup'ik study population. Functional genomic studies of the *CPT1A* variant and its modulation by n-3 PUFA intake, in addition to further investigation of the *CPT1A* gene in epidemiological studies among Arctic populations with variable n-3 PUFA intake will be required to validate the larger public health impact of these results. This study lays the foundation for future population-specific dietary recommendations based-on gene-diet interactions.

2.6 FIGURES

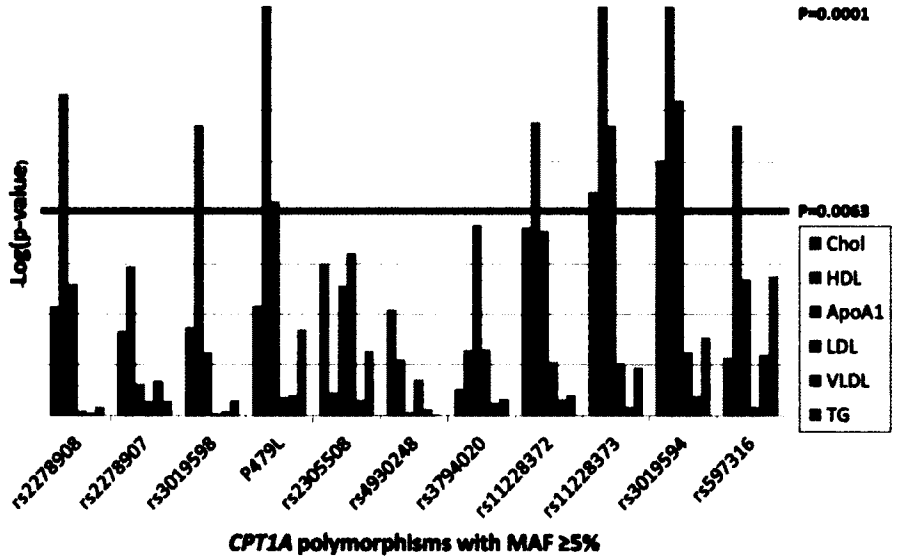

Figure 2.6.1: *CPT1A* polymorphisms with MAF ≥ 5% associated with obesity phenotypes. Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. The red line represents multiple test correction 8 tests that were estimated using the spectral decomposition of LD matrix (51). Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

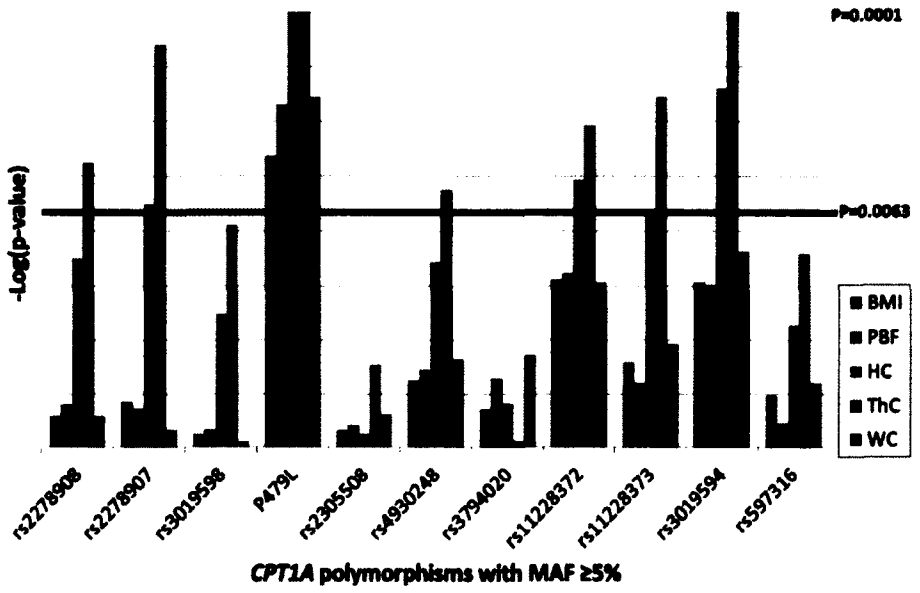

Figure 2.6.2: *CPT1A* polymorphisms with MAF ≥ 5% that are associated with obesity phenotypes. Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. The red line represents multiple test correction 8 tests that were estimated using the spectral decomposition of LD matrix (51). Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

2.7 TABLES

Table 2.7.1: Description of obesity-related traits in Yup'ik people[1,2].

Variables	Women	Men	p-values
No. of participants	601	539	
Age (yrs)	37.6 ± 17.3	35.9 ± 17.4	0.1113
Height (cm)	156.1 ± 6.2	167.7 ± 7.0	<0.0001
Weight (kg)	69.8 ± 16.7	73.0 ± 15.6	0.0003
Obesity Measures			
BMI (kg/m^2)	28.7 ± 6.8	25.9 ± 4.8	<0.001
Percentage Body Fat (%)	35.1 ± 8.9	21.1 ± 8.0	<0.0001
Waist Circumference (cm)	90.4 ± 15.9	89.2 ± 14.0	0.2102
Hip Circumference (cm)	104.1 ± 12.8	96.7 ± 8.3	<0.0001
Thigh Circumference (cm)	51.1 ± 5.6	50.2 ± 5.4	0.0071
Lipid Measures			
Cholesterol (mg/dL)	216.4 ± 44.6	208.9 ± 48.0	0.0088
HDL (mg/dL)	64.8 ± 18.5	56.2 ± 15.4	<0.0001
Apolipoprotein A1 (mg/dL)	170.8 ± 26.7	159.5 ± 26.8	<0.0001
LDL (mg/dL)	134.9 ± 36.5	135.9 ± 40.2	0.5500
VLDL (mg/dL)	16.9 ± 8.8	17.3 ± 10.6	0.4760
Triglyceride (mg/dL)	83.6 ± 42.8	84.8 ± 52.5	0.5967

[1] Values are reported as mean (± SD).

[2] Differences by gender are derived using student t-test.

Table 2.7.2: RBC nitrogen stable isotope ratio ($\delta^{15}N$) in Yup'ik people[1,2]

		Sex		Quartiles of $\delta^{15}N$			
	Total	**Women**	**Men**	**Q1**	**Q2**	**Q3**	**Q4**
No. of participants	1138	598	540	272	278	290	298
Mean ± SD (‰)	9.0 ± 1.5	9.1 ± 1.5	8.8 ± 1.5	7.3 ± 0.3	8.2 ± 0.2	9.1 ± 0.3	11.0 ± 1.0
Maximum	15.2	15.2	13.5	7.8	8.6	9.8	15.2
Minimum	6.2	6.3	6.2	6.2	7.8	8.6	9.8
Range (‰)	9.0	8.9	7.3	1.6	0.81	1.2	5.4

[1]Isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - ^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \cdot 1000‰$.

[2]The relationship between $\delta^{15}N$ and EPA follows the linear model: EPA (%RBC fatty acid) $=1.04 \cdot \delta^{15}N - 6.7‰$, as previously described for this population (37).

Table 2.7.3: *CPT1A* polymorphisms with MAF ≥0.05.

SNP[1]	Allele[2]	MAF[3]	Genotype[4]			Individuals[5] Genotyped	HWE[6] p-values
			AA	AB	BB		
rs2278908	C/T	0.06	2	40	1024	1066	0.1004
rs2278907	A/G	0.09	9	54	1074	1137	0.0270
rs3019598	G/A	0.05	2	37	1011	1050	0.0664
P479L (rs80356779)	A/G*	0.26	44	272	759	1075	0.1080
rs2305508	C/T	0.47	158	507	407	1072	0.7469
rs4930248	T/C	0.10	10	88	974	1072	0.0369
rs3794020	C/T	0.34	103	447	461	1011	1
rs2924697	G/C	0.28	1	533	452	986	0
rs11228372	G/A	0.14	7	158	906	1071	1
rs11228373	G/C	0.20	27	192	913	1132	0.0077
rs3019594	C/T	0.21	26	185	861	1072	0.0120
rs597316	G/C	0.05	1	42	1026	1069	0.4096

[1]Seattle SNPs Genome Variation Server on March 2008 (dbSNP build 126) Version 5.01.

[2]Major/ Minor allele.

[3]MAF computed using FREQ module in S.A.G.E.

[4]AA=Homozygous Recessive for minor allele, AB= Heterozygous, BB=Homozygous Dominant allele.

[5]Number of individuals genotyped for each *CPT1A* SNP.

[6]HWE p-values computed using PLINK.

*The P479L SNP is an A → G missense mutation at nucleotide position c.1436 of *CPT1A* which results in the substitution of a conserved proline (P479) for a leucine (L479) at position 479 in the CPT1A polypeptide.

Table 2.7.4: *CPT1A* polymorphisms associated with fasting lipids[1,2]

	Lipid Measures					
SNP	**Chol**	**HDL**	**ApoA1**	**LDL**	**VLDL**	**TG**
rs2278908	0.0842	**0.0007**	0.0501	0.9124	0.9449	0.8209
	(β=-0.9, SE=0.5)	**(β=-2.3, SE=0.7)**	(β=-1.5, SE=0.8)	(β=-0.1, SE=0.7)	(β=-0.1, SE=0.8)	(β=0.2, SE=0.8)
rs2278907	0.1478	0.0335	0.4893	0.7316	0.4551	0.7217
	(β=-0.6, SE=0.4)	(β=-1.0, SE=0.5)	(β=-0.4, SE=0.6)	(β=-0.2, SE=0.5)	(β=-0.4, SE=0.6)	(β=-0.2, SE=0.5)
rs3019598	0.1357	**0.0014**	0.2382	0.9588	0.9073	0.7069
	(β=-0.8, SE=0.6)	**(β=-2.2, SE=0.7)**	(β=-1.0, SE=0.8)	(β=0.0, SE=0.7)	(β=0.1, SE=0.8)	(β=0.3, SE=0.8)
P479L	0.0834	**0.0001**	0.0077	0.6629	0.6355	0.1407
(rs80356779)	(β=-0.4, SE=0.2)	**(β=-1.0, SE=0.3)**	(β=-0.8, SE=0.3)	(β=-0.1, SE=0.3)	(β=0.1, SE=0.3)	(β=0.4, SE=0.3)
rs2305508	0.0321	0.6052	0.0520	0.0250	0.7030	0.2284
	(β=-0.4, SE=0.2)	(β=0.1, SE=0.2)	(β=0.5, SE=0.2)	(β=-0.5, SE=0.2)	(β=-0.1, SE=0.2)	(β=-0.3, SE=0.2)
rs4930248	0.0912	0.2834	0.9245	0.4413	0.8735	0.9755
	(β=-0.6, SE=0.4)	(β=-0.5, SE=0.4)	(β=0.1, SE=0.5)	(β=-0.3, SE=0.4)	(β=-0.1, SE=0.5)	(β=0.0, SE=0.5)
rs3794020	0.5539	0.2261	0.0132	0.2244	0.7530	0.6833
	(β=-0.1, SE=0.2)	(β=0.3, SE=0.2)	(β=0.7, SE=0.3)	(β=-0.3, SE=0.2)	(β=0.1, SE=0.3)	(β=-0.1, SE=0.3)
rs11228372	0.0139	**0.0013**	0.0150	0.2982	0.6986	0.6246
	(β=-0.7, SE=0.3)	**(β=-1.2, SE=0.4)**	(β=-1.1, SE=0.4)	(β=-0.4, SE=0.4)	(β=-0.2, SE=0.4)	(β=0.2, SE=0.4)
rs11228373	**0.0063**	**<0.0001**	**0.0014**	0.3095	0.8199	0.3369
	(β=-0.7, SE=0.2)	**(β=-1.3, SE=0.3)**	**(β=-1.1, SE=0.4)**	(β=-0.3, SE=0.3)	(β=-0.1, SE=0.4)	(β=0.3, SE=0.3)
rs3019594	**0.0031**	**<0.0001**	**0.0008**	0.2377	0.6516	0.1691
	(β=-0.7, SE=0.2)	**(β=-1.4, SE=0.3)**	**(β=-1.2, SE=0.4)**	(β=-0.4, SE=0.3)	(β=0.2, SE=0.4)	(β=0.5, SE=0.3)
rs597316	0.2712	**0.0014**	0.0449	0.8213	0.2500	0.0417
	(β=-0.6, SE=0.6)	**(β=-2.2, SE=0.7)**	(β=-1.6, SE=0.8)	(β=-0.2, SE=0.7)	(β=0.9, SE=0.8)	(β=1.6, SE=0.8)

[1]Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Results are significant at p< 0.0063 and highlighted in bold. Multiple test correction for 8 tests for a phenotype was estimated using the spectral decomposition of LD matrix (51). Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

Table 2.7.5: *CPT1A* polymorphisms associated with obesity[1,2]

	Obesity Measures				
SNP	**BMI**	**PBF**	**HC**	**ThC**	**WC**
rs2278908	0.5137 (β=0.4, SE=0.6)	0.4006 (β=0.4, SE=0.5)	0.0179 (β=1.4, SE=0.6)	**0.0024 (β=2.1, SE=0.7)**	0.5103 (β=0.5, SE=0.8)
rs2278907	0.3778 (β=0.4, SE=0.42)	0.4339 (β=0.3, SE=0.4)	**0.0057 (β=1.2, SE=0.4)**	**0.0002 (β=1.9, SE=0.5)**	0.6956 (β=0.2, SE=0.5)
rs3019598	0.7691 (β=0.2, SE= 0.6)	0.6930 (β=0.2,SE= 0.5)	0.0578 (β=1.1, SE= 0.6)	0.0089 (β=1.9, SE=0.7)	0.9006 (β=0.1, SE=0.8)
P479L (rs80356779)	**0.0021 (β=0.7, SE=0.2)**	**0.0007 (β=0.7, SE=0.2)**	**<0.0001 (β=0.9, SE=0.2)**	**<0.0001 (β=1.2,SE= 0.3)**	**0.0006 (β=1.0, SE=0.3)**
rs2305508	0.7108 (β=-0.1, SE=0.2)	0.6326 (β=-0.1, SE=0.2)	0.7604 (β=0.1, SE=0.2)	0.1701 (β=0.3, SE=0.2)	0.4912 (β=-0.2, SE=0.2)
rs4930248	0.2369 (β=0.4, SE=0.4)	0.1874 (β=0.4, SE=0.3)	0.0191 (β=0.9, SE=0.4)	**0.0042 (β=1.3, SE= 0.5)**	0.1503 (β=0.7, SE=0.5)
rs3794020	0.4386 (β=-0.2, SE=0.2)	0.2276 (β=-0.2, SE=0.2)	0.3897 (β=-0.2, SE=0.2)	0.8763 (β=0.0, SE=0.2)	0.1390 (β=-0.4, SE=0.3)
rs11228372	0.0277 (β=0.7, SE=0.3)	0.0244 (β=0.6, SE=0.3)	**0.0034 (β=0.9, SE=0.3)**	**0.0011 (β=1.3, SE=0.4)**	0.0294 (β=0.9, SE=0.4)
rs11228373	0.1605 (β=0.4, SE=0.3)	0.2517 (β=0.3, SE=0.2)	**0.0063 (β=0.7, SE=0.3)**	**0.0006 (β=1.1, SE=0.3)**	0.1095 (β=0.5, SE=0.3)
rs3019594	0.0294 (β=0.6, SE=0.3)	0.0312 (β=0.5, SE=0.2)	**0.0005 (β=0.9, SE=0.3)**	**<0.0001 (β=1.3, SE=0.3)**	0.0154 (β=0.8, SE=0.3)
rs597316	0.3342 (β=0.6, SE=0.6)	0.6079 (β=0.3, SE=0.5)	0.0731 (β=1.1, SE=0.6)	0.0161 (β=1.8, SE=0.7)	0.2518 (β=0.9, SE=0.8)

[1]Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Results are significant at $p < 0.0063$ and highlighted in bold. Multiple test correction for 8 tests for a phenotype was estimated using the spectral decomposition of LD matrix (51). Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

Table 2.7.6: Obesity-related trait distribution within P479L genotypes [1,2,3]

Obesity Measures	L479/L479	L479/P479	P479/P479	p-value
BMI (kg/m^2)	26.2 (24.4-28.1)	28.0 (25.3-31.3)	31.1 (26.2-37.8)	**0.0021**
Percentage Body Fat (%)	27.8 (24.9-30.7)	30.0 (25.8-34.3)	33.7 (26.8-41.0)	**0.0007**
Waist Circumference (cm)	87.1 (82.7-91.9)	91.9 (85.2-99.8)	100.4 (88.1-116.4)	**0.0006**
Hip Circumference (cm)	98.2 (95.1-101.7)	101.6 (96.8-107.3)	106.6 (98.0-118.2)	**<0.0001**
Thigh Circumference (cm)	49.8 (48.0-51.7)	53.4 (50.6-56.4)	57.9 (52.9-63.5)	**<0.0001**

Lipids Measures	L479/L479	L479/P479	P479/P479	p-value
Cholesterol (mg/dL)	211.0 (197.4-225.6)	199.6 (181.6-219.8)	193.9 (166.5-266.8)	0.0834
HDL (mg/dL)	58.3 (53.3-64.1)	53.3 (47.2-60.6)	49.6 (41.1-60.7)	**0.0001**
Apolipoprotein A1 (mg/dL)	164.2 (155.2-173.9)	156.1 (144.2-169.1)	150.1 (132.4-170.5)	**0.0077**
LDL (mg/dL)	134.6 (122.4-147.8)	128.1 (111.6-146.4)	125.0 (99.7-155.1)	0.6629
VLDL (mg/dL)	15.1 (12.9-18.0)	15.8 (12.5-20.4)	17.2 (11.8-26.9)	0.6355
Triglyceride (mg/dL)	71.7 (61.7-84.2)	75.4 (60.9-95.5)	83.0 (58.6-125.1)	0.1407

[1] Values are reported as predicted mean (95% CI) obtained from ASSOC output.

[2] Association of the P479L (L479>P479) minor allele in the linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake.

[3]Results are significant at $p < 0.0063$ and highlighted in bold. Multiple test correction for 8 tests for a phenotype was estimated using the spectral decomposition of LD matrix (51).

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2.9 APPENDIX

Supplementary Table 2.9.1: Pairwise linkage disequilibrium (r^2) between *CPT1A* polymorphisms with MAF ≥0.05

SNP	rs2278908	rs2278907	rs3019598	P479L	rs2305508	rs4930248	rs3794020	rs2924697	rs11228372	rs11228373	rs3019594	rs597316
rs2278908		0.67	0.90	0.09	0.02	0.25	0.00	0.00	0.08	0.12	0.14	0.03
rs2278907	0.67		0.63	0.09	0.02	0.29	0.00	0.00	0.08	0.14	0.14	0.04
rs3019598	0.90	0.63		0.08	0.02	0.27	0.00	0.00	0.09	0.13	0.13	0.06
P479L	0.09	0.09	0.08		0.00	0.25	0.07	0.00	0.44	0.58	0.61	0.09
rs2305508	0.02	0.02	0.02	0.00		0.08	0.75	0.00	0.00	0.00	0.00	0.01
rs4930248	0.25	0.29	0.27	0.25	0.08		0.01	0.00	0.25	0.29	0.28	0.01
rs3794020	0.00	0.00	0.00	0.07	0.75	0.01		0.00	0.02	0.04	0.04	0.01
rs2924697	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.01	0.01	0.01	0.01
rs11228372	0.08	0.08	0.09	0.44	0.00	0.25	0.02	0.01		0.74	0.74	0.00
rs11228373	0.12	0.14	0.13	0.58	0.00	0.29	0.04	0.01	0.74		0.95	0.16
rs3019594	0.14	0.14	0.13	0.61	0.00	0.28	0.04	0.01	0.74	0.95		0.16
rs597316	0.03	0.04	0.06	0.09	0.01	0.01	0.01	0.01	0.00	0.16	0.16	

Pairwise linkage disequilibrium (r^2) was calculated using the hapfreq command in FBAT (42).

Supplementary Table 2.9.2: *CPT1A* polymorphisms associated with obesity-related traits with correction for BMI[1,2].

SNP	Chol	HDL	ApoA1
rs2278908	0.0738 (β=-1.0, SE=0.5)	**0.0007** (β=-2.1, SE=0.6)	0.04843 (β=-1.5, SE=0.6)
rs2278907	0.1294 (β=-0.6, SE=0.4)	0.0542 (β=-1.0, SE=1.0)	0.4939 (β=-0.4, SE=0.6)
rs3019598	0.125 (β=-0.9, SE=0.6)	**0.0009** (β=-2.1, SE=0.6)	0.1886 (β=-1.0, SE=0.8)
P479L	0.0489 (β=-0.4, SE=0.2)	**0.0023** (β=-0.7, SE=0.2)	0.0526 (β=-0.6, SE=0.3)
rs2305508	0.0247 (β=-0.4, SE=0.2)	0.628 (β=0.1, SE=0.2)	0.0453 (β=0.5, SE=0.2)
rs4930248	0.0743 (β=-0.6, SE=0.3)	0.4877 (β=-0.3, SE=0.4)	0.5947 **(β=0.3, SE=0.5)**
rs3794020	0.5055 (β=-0.1, SE=0.2)	0.2888 (β=0.2, SE=0.2)	0.0182 (β=0.6, SE=0.3)
rs11228372	0.0089 (β=-0.8, SE=0.3)	0.0097 (β=-0.9, SE=0.3)	0.0544 (β=-0.8, SE=0.4)
rs11228373	**0.0045** (β=-0.7, SE=0.2)	**<0.0001** (β=-1.1, SE=0.3)	**0.0025** (β=-1.0, SE=0.3)
rs3019594	**0.0018** (β=-0.8, SE=0.2)	**<0.0001** (β=-1.1, SE=0.3)	**0.0036** (β=-1.0, SE=0.4)
rs597316	0.2417 (β=-0.7, SE=0.6)	**0.0023** (β=-1.9, SE=0.6)	0.049 (β=-1.5, SE=0.8)

[1]Association of *CPT1A* SNPs in a linear regression model adjusted for age, sex, community membership, n-3 PUFA intake, and BMI. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Results are significant at $p < 0.0063$ and highlighted in bold. Multiple test correction for 8 tests for a phenotype was estimated using the spectral decomposition of LD matrix (51). Total cholesterol (Chol), high-density lipoprotein (HDL), and apolipoprotein A1 (ApoA1).

Supplementary Table 2.9.3: Association of P479L with HDL-Cholesterol with correction for PBF and WC

SNP	PBF[1]	WC[2]
P479L	**0.0032**	**0.0051**
	(β=-0.7, SE=0.2)	(β=-0.6, SE=0.2)

[1]Association of P479L in a linear regression model adjusted for age, sex, community membership, n-3 PUFA intake, and PBF. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Association of P479L in a linear regression model adjusted for age, sex, community membership, n-3 PUFA intake, and WC. Estimates of effect size (β) are reported using transformed phenotypes.

[3]Results are significant at $p < 0.0063$ and highlighted in bold. Multiple test correction for 8 tests was estimated using the spectral decomposition of LD matrix (51). High-density lipoprotein (HDL) cholesterol, percent body fat (PBF) and waist circumference (WC).

Supplementary Table 2.9.4: Interaction of *CPT1A* polymorphisms and n-3 PUFA intake on obesity-related traits[1].

	Obesity Measures					Lipid Measures					
SNP	**BMI**	**PBF**	**HC**	**ThC**	**WC**	**Chol**	**HDL**	**ApoA1**	**LDL**	**VLDL**	**TG**
rs2278908	0.6997	0.5860	0.5768	0.3308	0.7987	0.4877	0.5265	0.7858	0.7161	0.3411	0.8915
rs2278907	0.9279	0.8310	0.5648	0.5485	0.7775	0.1824	0.8763	0.1738	0.3854	0.2560	0.5888
rs3019598	0.6398	0.5636	0.6959	0.4958	0.7318	0.5335	0.4480	0.9069	0.7364	0.1764	0.6600
P479L	0.1717	0.1691	0.4205	0.0149	0.2248	0.6630	0.9256	0.4393	0.6964	0.2751	0.2157
rs2305508	0.8947	0.8565	0.5823	0.8936	0.8734	0.4015	0.0154	**0.0062**	0.5506	0.3590	0.3774
rs4930248	0.1699	0.4673	0.0818	0.0330	0.1034	0.8452	0.9753	0.8425	0.7743	0.7788	0.9990
rs3794020	0.6887	0.6889	0.7234	0.4303	0.4022	0.3630	**0.0032**	**0.0023**	0.2954	0.2715	0.2295
rs11228372	0.4352	0.5819	0.5190	0.1252	0.8422	0.6257	0.7445	0.3664	0.3263	0.9185	0.9420
rs11228373	0.4592	0.6869	0.3925	0.2150	0.8676	0.5157	0.9752	0.6635	0.3784	0.9065	0.8485
rs3019594	0.2141	0.4457	0.2387	0.0885	0.5671	0.5007	0.7437	0.2070	0.3959	0.9058	0.9589
rs597316	0.7939	0.9907	0.5994	0.7873	0.9745	0.3530	0.2152	0.2169	0.6192	0.1509	0.3037

[1]Results are significant at $p < 0.0063$ and highlighted in bold. Multiple test correction for 8 tests for a phenotype was estimated using the spectral decomposition of LD matrix (51). Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC), waist circumference (WC), total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL), triglycerides (TG).

GENETIC POLYMORPHISMS IN *SLC2A4* and *SCD* ARE ASSOCIATED WITH VARIATION OBESITY-RELATED PHENOTYPES IN YUP'IK PEOPLE[2]

3.1 ABSTRACT

OBJECTIVE: Regular consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) has been associated with protection from obesity-related diseases, in part, by altering the activity of genes involved with lipid and glucose metabolism. Stearoyl CoA desaturase (*SCD*), insulin sensitive glucose transporter (*SLC2A4*), and steroyl regulatory element binding protein (*SREBF1)* are obesity candidate genes that are transcriptionally regulated by n-3 PUFA intake. Although polymorphisms in *SCD, SLC2A4,* and *SREBF1* have been associated with obesity-related phenotypes, the extent to which these genetic associations are modified by interactions with n-3 PUFA intake remains unknown. DESIGN: Obesity and lipid-related phenotypes were measured in a cross-sectional sample of Yup'ik individuals (n=1080) enrolled in the Center of Alaska Native Health Research (CANHR) study. A comprehensive list of thirty-three single nucleotide polymorphisms within or near *SCD, SLC2A4,* and *SREBF1* were tested for association with outcomes of interest in linear models accounting for familial correlations. Dietary intake of n-3 PUFAs was estimated using nitrogen stable isotope ratios ($\delta^{15}N$) of red blood cells (RBC). RESULTS: *SCD* (rs11190480 and rs2167444) polymorphisms were positively associated with ApoA1 levels (p=0.006 and p=0.004, respectively). *SLC2A4* polymorphisms (rs5415 and rs5435) were associated lower fasting HDL-cholesterol (p=0.001 and p=0.003, respectively) as well as increased hip circumference (p=0.005 and p=0.027, respectively). CONCLUSION: Our results indicate that polymorphisms in *SCD* and *SLC2A4* are associated with obesity-related phenotypes in Yup'ik people; however we did not detect

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[2] Lemas, DJ, Klimentidis YC, Wiener HW, O'Brien DM, Hopkins S, Stanhope KL, Havel PJ, Allison DB, Fernandez, JR, Tiwari HK, Boyer BB. 2012. Genetic Polymorphisms in *SLC2A4* and *SCD* are Associated with Variation Obesity-Related Phenotypes in Yup'ik People. Prepared for submission to *The International Journal of Obesity*.

significant n-3 PUFA interactions between SNPs in *SCD, SLC2A4,* and *SREBF1* that modified genetic associations with obesity phenotypes.

3.2 INTRODUCTION

In the United States, nearly 70% of adults are overweight and more than half of these individuals are obese [1]. Accumulation of excess body fat increases the risk of developing obesity-related diseases that include type 2 diabetes (T2D), cardiovascular disease and cancer [2]. Moderate weight loss (10% of body weight), as a treatment for obesity, has been shown to attenuate the risk of developing obesity-related diseases [3], however weight loss is difficult to maintain [4]. Given the increasing public health burden attributed to obesity and the difficulty achieving and maintaining weight loss [5–7], it is important to define genetic and environmental factors that influence the development of obesity and lipid-related phenotypes.

Regular consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) may reduce adiposity in humans [8], in part, by regulating the activity of three genes: stearoyl CoA desaturase (*SCD*) [9], insulin sensitive glucose transporter (*SLC2A4*) [10], and steroyl regulatory element binding protein (*SREBF1)* [11]. SCD is the rate-controlling enzyme catalyzing the biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acid substrates [12]. *SLC2A4* codes for a specialized insulin- stimulated glucose transporter that regulates glucose uptake in skeletal muscle, heart, and adipose tissue [13]. *SREBF1* encodes a transcription factor that plays a critical role in energy homeostasis by promoting glycolysis, lipogenesis, and adipogenesis [14]. Although whole-genome linkage studies have implicated *SCD*, *SLC2A4*, and *SREBF1* as obesity candidate genes [15–17], the extent to which n-3 PUFA intake modifies the association between polymorphisms in *SCD*, *SLC2A4*, and *SREBF1* and obesity-related phenotypes is unknown.

Given the widely varying intake of n-3 PUFA in this study population of Yup'ik people (30-fold range), the proposed health benefit of eating a diet rich in n-3 PUFAs, and the availability of a precise biomarker for n-3 PUFA intake ($\delta^{15}N$; [18]); we tested polymorphisms in obesity candidate genes regulated by n-3 PUFA for association with

obesity-related phenotypes in Yup'ik people and investigated the extent to which these associations were modified by n-3 PUFA. Specifically, the candidate genes (*SCD*, *SLC2A4*, and *SREBF1*) examined in this study were identified in whole-genome linkage analysis [16,17,19–23] and tested in Yup'ik participants from the Center for Alaska Native Health Research (CANHR) study.

3.3 METHODS

3.3.1 PARTICIPANTS AND METHODS

The CANHR study investigates genetic, behavioral, and dietary risk and protective factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik people [24]. A community-based participatory research framework guides all CANHR investigations, such that participant ascertainment is open to all members of the community meeting a specified age minimum. Recruitment of Yup'ik participants was initiated in 2003 and continues in 11 Southwest Alaska communities. All residents 14 years of age and older are invited to participate and the resulting distribution of age in our study sample reflects the age distribution among eligible participants according to 2000 U.S. census data. Participants signed informed-consent documents before entering the study using protocols that were approved by the University of Alaska Institutional Review Board, the National and Alaska Area Indian Health Service Institutional Review Boards, and the Yukon-Kuskokwim Health Corporation Human Studies Committee. The analyses in this report were performed on 1080 non-pregnant Yup'ik participants with ages that ranged between 14 and 94 years at the time of enrollment.

3.3.2 ANTHROPOMETERIC AND BIOCHEMICAL MEASUREMENT

Trained staff obtained anthropometric measurements using protocols from the NHANES III Anthropometric Procedures Manual [25] as previously described [26]. These measurements included height, weight and 4 circumferences (waist, hip, triceps, and thigh). Percent body fat (PBF) was measured by electrical bioimpedance using a Tanita

TBF-300A body fat analyzer (Tanita Corp, Arlington Heights, IL, U.S.A.). Blood samples were collected from participants after an overnight fast, and lipoprotein measures including total cholesterol, HDL- cholesterol, LDL-cholesterol, VLDL cholesterol, apolipoprotein A1, and plasma triglycerides levels were assayed as previously described [26].

3.3.3 BIOMARKER FOR MARINE n-3 PUFA INTAKE

n-3 PUFA intake was assessed in Yup'ik individuals using the nitrogen stable isotope ratio of red blood cells (RBC) as previously described [18]. RBC aliquots were autoclaved for 20 minutes at 121°C to destroy blood-borne pathogens, and samples were weighed into 3.5 x 3.75 mm tin capsules and freeze dried to a final mass of 0.2 - 0.4 mg. Samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio mass spectrometry, using a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo-Finnigan Inc., Breman, Germany). Isotope ratios are analyzed relative to IAEA-certified reference materials calibrated to atmospheric nitrogen, for which $^{15}N/^{14}N = 0.0036765$. By convention and for ease of interpretation, isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N\text{sample} - {}^{15}N/^{14}N\text{standard})/(^{15}N/^{14}N\text{standard})] \cdot 1000‰$. We concurrently prepared and ran multiple laboratory standards (peptone, $\delta^{15}N = 7.00$) to assess analytical accuracy and precision; these were analyzed after every eighth sample and gave values of $\delta^{15}N = 7.01 \pm 0.24‰$ (mean ± SD). The range of isotopic variation in our dataset (9‰) was very large relative to analytical precision (0.2‰). We modeled the effects of n-3 PUFA intake as a categorical variable, which is hereafter referred to as $\delta^{15}N$.

3.3.4 SNP SELECTION AND GENOTYPING

A comprehensive list of DNA variants were selected for genotyping within and near (5 kb upstream and 5 kb downstream) the *SCD*, *SLC2A4* and *SREBF1* genes collected from HapMap data, release #3, National Center for Biotechnology Information (NCBI) B36, dbSNP 126 [27]. Given that no publically available genotypic information exists on Yup'ik people, we referenced the Caucasian (CEU) and Han Chinese (CHB) populations in HapMap using the SeattleSNPs database (http://pga.mbt.washington.edu/) to identify potential genetic variants that may be common in our study population. A set of 27 maximally informative tagging SNPs (tSNPs) within or near *SCD*, *SLC2A4* and *SREBF1*were identified to represent common linkage disequilibrium clusters with the LDselect algorithm as implemented in the MultiPop-TagSelect program using thresholds of r^2=0.90 and minor allele frequency >1% [28,29]. We chose to relax our MAF criteria to include SNPs with MAF >0.01 in order to genotype tagging SNPs in the *SCD*, *SLC2A4* and *SREBF1* genes which may be common (MAF≥0.05) in Yup'ik people despite being rare (MAF<0.05) in CEU and CHB populations. Additional SNPs in *SCD* (rs41290540 and rs2167444) were selected based on previous SNP associations with obesity and lipid-related phenotypes [30,31]. Additional SNPs in *SLC2A4* (rs2654185, SNP 12 and SNP 13) were selected based on previous associations with HbA1c [32] and resequencing candidate genes in participants enrolled in the CANHR study. Specifically, 30 unrelated Yup'ik participants enrolled in the CANHR study were selected based on extreme BMI measures (15 participants with lowest BMI and 15 participants with highest BMI) and *SLC2A4* resequencing was limited to all protein coding regions, intron-exon boundaries, and 1,000 bp of 5′ and 300 bp of 3′ flanking DNA (Agencourt; Beverly, MA). Sequence information used to generate primers for SNP 12 and SNP 13 in *SLC2A4* is presented in **Supplementary Table 3.9.1**. Our final genotyping list included a total of 34 SNPs in *SCD* (13 SNPs), *SLC2A4 (*13 SNPs), and *SREBF1* (8 SNPs) that were genotyped using allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight spectrometry on a Sequenom iPLEX platform at the Broad Institute [33]. Linkage disequilibrium (LD) among SNPs in

each gene was based on pairwise haplotype frequencies calculated using the hapfreq command in the FBAT program [34].

3.3.5 QUALITY CONTROL OF PHENOTYPIC AND GENOTYPIC DATA

Simple linear models were fit to each of the outcome variables using all of the covariates (age, sex, community membership) included in the association models, and the distributions of the residuals were examined for normality with the R statistical programming language (v2.10.1, R Development Core, 2009). We considered a series of transformations (square root, log, inverse) to improve normality and the Box-Cox transformation [35] was identified as the best procedure for phenotypes whose residuals did not follow a normal distribution. Family data was extracted from the Progeny database (Progeny Software LLC, South Bend, IN, U.S.A.) and merged into a single extended pedigree using PedMerge [36]. Genotypic data were tested for Mendelian inconsistencies using PEDCHECK [37]. In this sample, Illumina IV linkage panel (Illumina, Inc., San Diego, CA, USA) genotypes were available from ongoing linkage study and were used to calculate ancestry using principal components analysis (PCA) program in the EIGENSTRAT analysis package [38]. The second PC discriminated the individuals in the study into two groups that correspond to the proximity of the community to the coast. Based on this observation, we defined a dichotomous community group variable. We assessed Hardy-Weinberg equilibrium (HWE) for each SNP using an algorithm that accounts for relationships among individuals [39]. Allele frequencies for each SNP were computed using the FREQ module in the program Statistical Analysis for Genetic Epidemiology (S.A.G.E., 2009) in the entire dataset. The present study restricted genetic analysis to SNPs in *SCD*, *SLC2A4*, and *SREBF1* that had a MAF $\geq$ 5% and did not deviate from HWE. HWE multiple test correction to control the familywise error rate was determined for each gene using spectral decomposition of linkage disequilibrium (LD) matrices generated for each pair of genotyped markers [40,41]. Given these criteria, the HWE multiple test correction was estimated to be 12 tests for *SCD* ($\alpha \leq 0.004$), 6 tests for *SLC2A4* ($\alpha \leq 0.008$), and 6 tests for *SREBF1* ($\alpha \leq 0.008$).

3.3.6 ASSOCIATION ANALYSIS

Each SNP was tested for association with obesity and lipid-related phenotypes using the program ASSOC [42] in the S.A.G.E software package which can incorporate complex pedigree data, covariates and interactions into association analysis. We included both demographic (age, community, and sex) and environmental variables ($\delta^{15}N$) in the ASSOC analysis. Likelihood ratio statistics were calculated to compare 3 nested models and test the null hypothesis of no association between SNPs and obesity and lipid-related phenotypes after including demographic and environmental covariates. Effect sizes (β) were extracted from linear models adjusted for demographic and environmental covariates to illustrate the change in transformed phenotypes according to each copy of the minor allele tested.

Model 1 included baseline covariates (age, sex, community membership, and $\delta^{15}N$ quartiles); Model 2 included baseline covariates and SNP to test for an additive genetic effect of SNP (defined as the number of minor alleles); Model 3 included baseline covariates, the additive genetic effect of SNP, $\delta^{15}N$ quartiles, and interactions between the additive genetic effect and $\delta^{15}N$ quartiles. Note that the model 3 is the only model to test directly gene-diet interaction under the null hypothesis. We have treated each phenotype being tested as representing a separate family of null hypotheses and correct for the number of tests within each family [43]. P-values were compared to the conventional significance threshold ($p<0.05$), as well as significance thresholds adjusted for multiple test correction. Multiple test correction for genetic analysis was used to control the familywise error rate that was calculated according to the number of non-redundant SNPs with MAF ≥ 0.05. Given the correlation among neighboring genetic markers, the effective numbers of non-redundant SNPs for *SCD* (12 tests; $\alpha \leq 0.004$), *SLC2A4* (6 tests; $\alpha \leq 0.008$), and *SREBF1* (6 tests; $\alpha \leq 0.008$) were estimated using spectral decomposition of LD matrices [40,41].

3.4 RESULTS

3.4.1 CHARACTERISTICS OF YUP'IK PARTICIPANTS

Descriptive statistics on Yup'ik women and men enrolled in the CANHR study are presented in **Table 3.7.1**. In general, Yup'ik women had significantly greater levels of adiposity (BMI, PBF, and HC) and higher fasting HDL-cholesterol and ApoA1 levels relative to Yup'ik men ($p<0.0001$).

3.4.2 DISTRIBUTION OF $\delta^{15}N$ IN STUDY POPULATION

We assessed n-3 PUFA intake in 1080 Yup'ik participants using RBC $\delta^{15}N$ as a biomarker of EPA and DHA intake. Summary statistics are grouped by gender and $\delta^{15}N$ quartiles and reported in **Table 3.7.2**. The mean $\delta^{15}N$ value in this study was 8.9‰, and ranged from 6.2‰ to 15.2‰. According to the linear relationship between RBC $\delta^{15}N$ and RBC EPA described elsewhere for this study population [18], the corresponding mean EPA (%RBC fatty acids) was 2.6%. Measurement of $\delta^{15}N$ by gender yielded means of 9.1‰ for females and 8.8‰ for males. The mean RBC $\delta^{15}N$ values by quartile were: 7.3‰, 8.2‰, 9.1‰, 11.0‰ in groups 1-4, respectively. These values correspond to EPA (% RBC fatty acids) group means of: 0.9%, 1.9%, 2.8%, and 4.7% [18]. The standard deviation of $\delta^{15}N$ in this sample did not differ according to gender (1.5‰ for both females and males).

3.4.3 GENETIC VARIATION IN *SCD*, *SLC2A4*, AND *SREBF1*

A comprehensive list of thirty-three SNPs in *SCD*, *SLC2A4* and *SREBF1* were genotyped in 1080 Yup'ik participants that had a mean success rate of 94.7% (range 70.6-94.6%). Genotyping in this study revealed that 6 SNPs were monomorphic (*SLC2A4*: SNP12, SNP13, rs222842 and rs3744404; *SREBF1*: rs11653007 and rs1108511), 5 SNPs had MAF <0.05 and >0.01 (*SCD*: rs10883463; *SLC2A4*: rs2073476, rs5412, and rs222847; *SREBF1*: rs4925118), and 23 SNPs had MAF $\geq$ 0.05. The rs41290540 SNP in *SCD* (MAF=0.12) was the only polymorphism with MAF $\geq$0.05 that deviated significantly from HWE proportions (p=0.0002) and was excluded from the

analysis. The genetic analyses in this study included the 11 SNPs in *SCD*, 6 SNPs in *SLC2A4*, and 6 SNPs in *SREBF1* with MAF $\geq$ 0.05 that did not deviate from HWE proportions (**Table 3.7.3**). Multiple test correction [40,41] was determined for each gene and estimated that 7 of the 11 markers in *SCD* (per-test α level was <0.007), all 6 markers in *SCL2A4* (per-test α level was <0.008), and 4 of the 6 markers in *SREBF*1 (per-test α level was <0.013) were non-redundant genetic markers.

3.4.4 SNP ASSOCIATIONS WITH LIPID PHENOTYPES

Figure 3.6.1 and **Table 3.6.4 presents** SNPs in *SCD*, *SLC2A4,* and *SREBF1* with MAF $\geq$0.05 that were associated with lipid phenotypes. Notably, rs11190480 and rs2167444 in *SCD* were in strong LD (r^2=0.95, **Supplementary Table 3.9.2**) and positively associated with ApoA1 levels (p=0.006 and p=0.004, respectively). Additional *SCD* SNPs (rs11557927 p=0.008 and rs7849 p=0.008) were very close to passing multiple test correction and several *SCD* SNPs (rs11190480, rs115579277, and rs7849) were nominally associated increased HDL-cholesterol (p=0.029, p=0.045, and p=0.048, respectively). The rs5415 and rs5435 polymorphisms in *SLC2A4* were in moderately strong LD (r^2=0.74, **Supplementary Table 3.9.3**) and negatively associated with fasting ApoA1 levels (p=0.005 and p=0.003, respectively) and HDL-cholesterol (p=0.001 and p=0.003, respectively). Additional *SLC2A4* SNPs (rs5417 p=0.009 and rs3744405 p=0.009) were very close to passing multiple test correction and an *SCD* SNP (rs2654185) was nominally associated (p=0.016) with increased ApoA1 levels. We found *SREBF1* SNPs (rs2297508, rs2282180, and rs8066560) in strong LD (r^2=0.93-1.0); **Supplementary Table 3.9.4**) that were nominally associated with fasting measures of LDL-cholesterol (p=0.029, p=0.023, and p=0.014, respectively) and a *SREBF1* SNP (rs8066560) associated with total cholesterol (p=0.014). Finally, we detected significant interactions between *SCD* SNPs (rs2167444, p=0.0068 and rs11190480, p=0.0047) and n-3 PUFA intake (**Supplementary Table 3.9.5**); however after correction for HDL-cholesterol these results were only nominally significant (rs2167444, p=0.0494 and rs11190480, p=0.0113) (**Supplementary Table 3.9.6**).

3.4.5 SNP ASSOCIATIONS WITH OBESITY PHENOTYPES

Figure 3.7.2 and **Table 3.8.5** presents SNPs in *SCD*, *SLC2A4,* and *SREBF1* with MAF ≥0.05 that were associated with obesity phenotypes. Notably, rs5415 in *SLC2A4* was positively associated with hip circumference (p=0.005) and nominally associated with increased thigh circumference (p=0.034). Additional *SLC2A4* SNPs (rs2654185, rs5417, and rs5435) were nominally associated with increased hip circumference (p=0.011, p=0.016, and p=0.027, respectively) and increased thigh circumference (p=0.012, p=0.020, and p=0.040, respectively). Furthermore, SNPs (rs1502593 and rs3071) in *SCD* were nominally associated with reduced BMI (p=0.035 and p=0.043, respectively) and WC (p=0.028 and p=0.028, respectively) in our study. We did not observe SNPs in *SREBF1* that were associated with obesity phenotypes. Furthermore, we did not detect significant n-3 PUFA interactions between SNPs in *SCD, SLC2A4,* and *SREBF1* that modified genetic associations with obesity phenotypes. All interactions results for obesity phenotypes are presented in **Supplementary Table 3.9.7.**

3.5 DISCUSSION

Regular consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) has been associated with protection from obesity [8], in part, by altering the expression of genes implicated in obesity pathophysiology. In this study we comprehensively evaluated SNPs within or near *SCD*, *SLC2A4*, and *SREBF*1 for association with obesity and lipid-related phenotypes and explored whether these genetic associations were modified by interactions with n-3 PUFA intake in a study population with up to a 30-fold variation in consumption of n-3 PUFAs [18]. Our results demonstrate that two *SCD* polymorphisms (rs1190480 and rs2167444) were associated with elevated fasting ApoA1 levels. Additionally, *SLC2A4* polymorphisms (rs2654185, rs5415, rs5417 and rs5435) were associated lower fasting HDL-cholesterol and increased hip circumference and thigh circumference. Taken together, our results indicate that polymorphisms in *SCD* and *SLC2A4* are associated with variation in obesity and lipid-related phenotypes in Yup'ik

people; however we did not detect significant n-3 PUFA interactions between SNPs in *SCD, SLC2A4,* and *SREBF1* that modified genetic associations with obesity phenotypes.

Previous studies that have examined *SCD* polymorphisms associated with obesity-related phenotypes have produced mixed results [30,31,44]. Liew *et al.* failed to detect *SCD* polymorphisms associated with type 2 diabetes (T2D) traits, BMI, or waist-hip-ratio in a large European case-control study [30]. Gong *et al.* reported that *SCD* genetic variation was associations with metabolic syndrome (MetS) in a Costa Rican cohort [44] and Warensjö *et al.* reported *SCD* polymorphisms were associated with insulin sensitivity, reduced BMI and smaller waist circumference in Swedish men [31].

We did not evaluate the contribution of *SCD* polymorphisms to T2D and MetS given the historically low prevalence of T2D (<3% in Yup'ik people) [24] and MetS (8.6 % in men and 19.8% in women) [26] observed among Yup'ik people. Nevertheless, we investigated the association between *SCD* polymorphisms and three of the five traits associated with MetS (HDL-cholesterol, triglycerides and WC) and we did not observe any significant associations. Furthermore, the *SCD* polymorphism (rs1502593) associated with MetS in Costa Rican adults [44] was nominally associated with reduced BMI (p=0.035) and WC (p=0.028) in our Yup'ik study population. The *SCD* polymorphism (rs2167444) associated with improved insulin sensitivity and reduced adiposity in Swedish men [31], was positively associated with fasting ApoA1 levels, but not HDL-cholesterol, in our Yup'ik study population. Taken together, our *SCD* genetic association results are generally consistent with the findings of Warensjö *et al.* and demonstrate that *SCD* polymorphisms are associated with elevated fasting ApoA1 levels and reduced obesity Yup'ik people.

In mice, SCD1 *(*mouse *SCD* homolog*)* deficiency is associated with increased energy expenditure [45], reduced adiposity [46], increased insulin sensitivity [47], protection against hypertriglyceridemia [48], and elevated HDL levels [48]. Consumption of polyunsaturated fatty acids transcriptionally regulates SCD [49] by repressing *SCD* mRNA levels in the liver [9] and adipocytes [50]. Our results demonstrate that *SCD* polymorphisms were associated with reduced BMI, smaller hip circumference and elevated fasting

ApoA1 levels, the major apolipoprotein found in HDL-cholesterol, in a population with widely varying n-3 PUFA intake. Although functional evidence suggests consumption of n-3 PUFA transcriptionally regulates SCD activity, our analyses did not find that n-3 PUFA intake modified genetic associations between *SCD* polymorphisms and obesity phenotypes in this Yup'ik study population.

Interrogation of *SLC2A4* genetic variation has largely focused on identifying polymorphisms associated with T2D status [51–61]. Early studies using restriction fragment length polymorphism analyses did not detect *SLC2A4* (also known as *GLUT4* [*Glucose transporter type 4*] gene) associations with T2D in European whites [51,60,61], African Americans [57,61], Chinese [61], Japanese [61] and Asian Indians [61]. Similarly, analysis of single-strand conformation polymorphism analysis in Welsh [60], British [52] and Italian [53] cohorts failed to detect *SLC2A4* polymorphisms associated with T2D. Finally, linkage analysis in a French cohort [55] and direct sequence analysis in a mixed US sample [54] did not reveal significant *SLC2A4* variations associated with T2D status.

Candidate gene studies that have examined *SLC2A4* polymorphisms for association with obesity-related phenotypes have produced mixed results [56,58,59,61,62]. BMI was not associated with *SLC2A4* polymorphisms in a cohort of German children [62] or case-control studies for T2D [51,56,58,61]. In contrast, *SLC2A4* polymorphisms were associated with fasting insulin levels in a South Indian cohort [63] and fasting HbA1c levels in a cohort of Japanese men [32]. Taken together, these studies suggest that genetic variation in *SLC2A4* may contribute to changes in T2D-related traits.

Our results demonstrate that *SLC2A4* polymorphisms (rs5435 and rs2654185) previously associated with T2D-related traits [32,63], are associated with lower fasting HDL-cholesterol ($p=0.003$ and $p=0.016$, respectively) in Yup'ik people. Additionally, the *SLC2A4* polymorphism (rs5435) reported by Bodhini *et al.* to be positively associated with fasting insulin levels, was in strong LD ($r^2=0.74$) with a *SLC2A4* polymorphism (rs5415) associated with reduced fasting HDL-cholesterol ($p=0.001$) and ApoA1 levels ($p=0.005$) as well as increased hip circumference ($p=0.005$) in our study population.

Taken together, our results extend the findings of Bodhini *et al.* and Xi *et al.* by showing that *SLC2A4* genetic variation is associated with T2D and obesity-related traits.

SREBF1 polymorphisms have been associated with atherogenic lipid profiles [64], plasma adiponectin levels [65], HIV-related hyperlipoproteinaemia [66], odds of developing T2D [67–69], extreme obesity [67], as well as variation in total cholesterol [68,70,71] and LDL cholesterol [68,69,72]. The non-synonymous G952G (rs2297508) variant in *SREBF1* has been associated with T2D [65,67,69,73,74], obesity [67], and cholesterol metabolism [66,70,71,73]. Interestingly, two previous studies have failed to observe significant associations between G952G (rs2297508) and total and LDL-cholesterol [67,73]. Our analysis did not evaluate *SREBF1* polymorphisms for association with T2D-related traits; however we did detect nominally significant associations with fasting lipid phenotypes. We observed *SREBF1* SNPs (rs8066560 and rs2282180) including the G952G (rs2297508) variant nominally ($p<0.05$) associated with elevated fasting LDL-cholesterol levels in our study population. Interestingly, the rs8066560 polymorphism was nominally associated with elevated fasting total cholesterol levels ($p=0.025$) and in strong LD with G952G (rs2297508) ($r^2=0.93$). Our results provide preliminary evidence of association between the non-synonymous G952G (rs2297508) variant in *SREBF1* and fasting LDL-cholesterol levels in Yup'ik people.

Our gene-by-diet analysis revealed n-3 PUFA intake modified the association between *SCD* polymorphisms (rs1190480 and rs2167444) and fasting total cholesterol levels in Yup'ik people. Relative to individuals homozygous for the major allele, our analysis indicated that participants carrying the minor allele for either SNP (rs1190480 and rs2167444) had higher fasting cholesterol levels for any level of n-3 PUFA intake with the exception of n-3 PUFA intake at quartile 3. Although statistically significant, it is unclear why individuals homozygous for the *SCD* minor alleles would be expected to have lower fasting cholesterol levels at moderately high levels of n-3 PUFA intake (quartile 3). Given these *SCD* (rs1190480 and rs2167444) polymorphisms were associated with HDL-cholesterol levels, we adjusted these analyses for HDL-cholesterol and found that total cholesterol levels were no longer significantly modified by

interactions with *SCD* polymorphisms and n-3 PUFA intake (**Supplementary Table 3.9.6**). Taken together, these results suggest the interaction of *SCD* polymorphisms and n-3 PUFA intake on total cholesterol levels may, in part, be mediated by changes in fasting HDL-cholesterol levels.

Our Yup'ik study population was ideally suited to investigate the impact of n-3 PUFA [75,76] and genetic factors [77] on obesity and lipid-related phenotypes given the 30-fold range of n-3 PUFA consumption in this population [78], the traditional beliefs and scientific evidence that subsistence foods rich in n-3 PUFAs are healthy, and the $\delta^{15}N$ variable which can precisely estimate n-3 PUFA intake in large epidemiologic samples using nitrogen stable isotope ratios from red blood cell [18]. The strengths of this study include a sample size large enough to detect significant SNP associations and a statistical approach that accounts for relationships among participants while also allowing for covariates [42]. Factors that may account for the differences in results reported by the present study and the other investigations may include, but are not limited to, small sample size, population stratification and differences in statistical analysis [79,80].

In summary, we have shown that polymorphisms within or near *SCD*, *SLC2A4*, *SREBF1* are associated with obesity and lipid-related phenotypes in a study population with widely varying n-3 PUFA intake, however we did not detect significant n-3 PUFA interactions between SNPs in *SCD*, *SLC2A4*, and *SREBF1* that modified genetic associations with obesity phenotypes. Additional genomic studies will be required in larger cohorts with variable intake of n-3 PUFAs in order to determine the validity and public health implications of our findings.

3.6 FIGURES

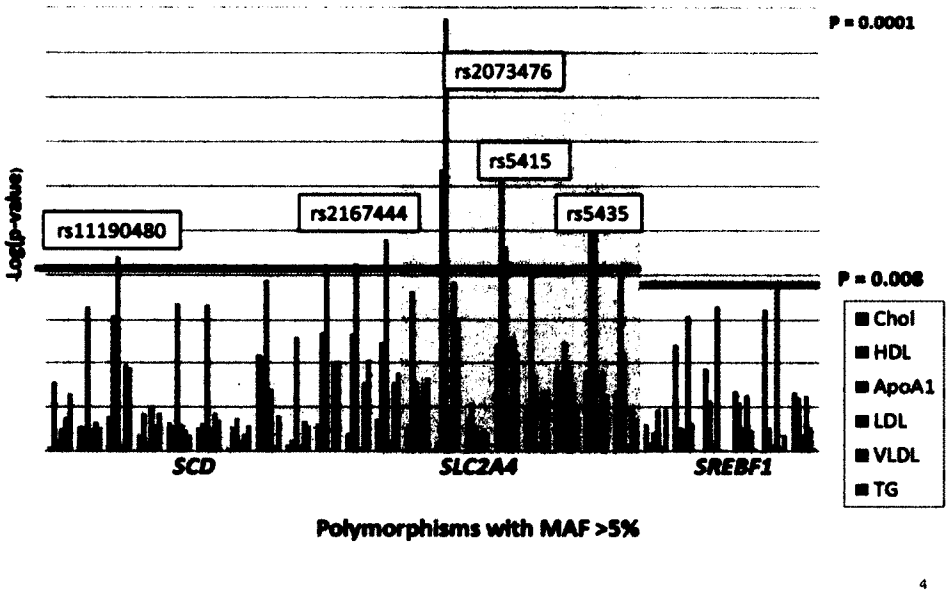

Figure 3.6.3: *SCD, SLC2A4* and *SREBF1* polymorphisms with MAF ≥ 5% that are associated with lipid phenotypes.

Association of SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. The red line represents multiple test correction adjusted for each gene that was estimated using the spectral decomposition of LD matrix (50). Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

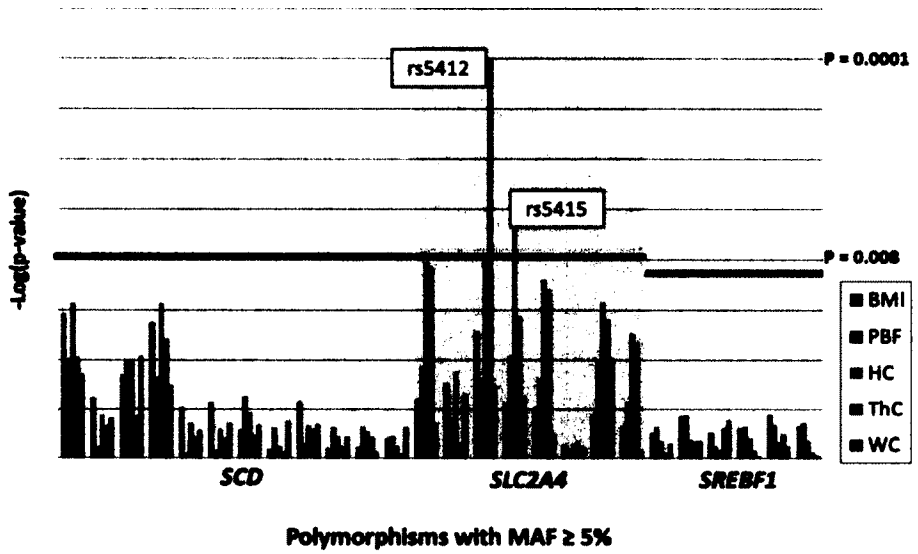

Figure 3.6.4: *SCD, SLC2A4* and *SREBF1* polymorphisms with MAF ≥ 5% that are associated with obesity phenotypes.

Association of SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. The red line represents multiple test correction adjusted for each gene that was estimated using the spectral decomposition of LD matrix (50). Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

3.7 TABLES

Table 3.7.1: Description of Yup'ik people according to gender[1,2].

Variables	Women	Men	p-values
No. of participants	566	517	
Age (yrs)	40.2 ± 3.6	38.6 ± 2.6	0.1286
Obesity Measures			
BMI (kg/m^2)	28.7 ± 5.6	26.3 ± 5.6	< 0.0001
Percentage Body Fat (%)	42.6 ± 1.4	27.6 ± 1.1	< 0.0001
Waist Circumference (cm)	87.9 ± 21.6	87.5 ± 21.5	0.6646
Hip Circumference (cm)	105.4 ± 43.0	98.7 ± 43.0	< 0.0001
Thigh Circumference (cm)	51.0 ± 21.4	50.3 ± 21.3	0.0239
Lipid Measures			
Cholesterol (mg/dL)	198.5 ± 67.8	191.3 ± 67.0	0.0122
HDL (mg/dL)	66.4 ± 20.0	57.0 ± 19.8	< 0.0001
Apolipoprotein A1 (mg/dL)	175.7 ± 60.4	163.1 ± 59.9	< 0.0001
LDL (mg/dL)	128.9 ± 1.1	129.4 ± 1.0	0.8213
VLDL (mg/dL)	15.0 ± 1.0	15.1 ± 1.0	0.8424
Triglyceride (mg/dL)	74.6 ± 23.5	73.8 ± 23.1	0.6319

[1] Values are reported as mean (± S.E.) predicted from linear model accounting for familial correlations.

[2] P-values for differences by gender are derived using student t-test.

Table 3.7.2: Distribution of the RBC nitrogen stable isotope ratio ($\delta^{15}N$) in Yup'ik people[1,2]

		Sex		Quartiles of $\delta^{15}N$[**]			
	Total	Women	Men	Q1	Q2	Q3	Q4
No. of participants	1080	564	515	259	262	273	285
Mean ± SD (‰)	8.9 ± 1.5	9.1 ± 1.5	8.8 ± 1.5	7.3 ± 0.3	8.2 ± 0.2	9.1 ± 0.3	11.0 ± 1.0
Maximum	15.2	15.2	13.5	7.8	8.6	9.8	15.2
Minimum	6.2	6.4	6.2	6.2	7.8	8.6	9.8
Range (‰)	9	8.8	7.3	1.6	0.8	1.2	5.4

[1]Isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(\ {}^{15}N/^{14}N_{standard})] \cdot 1000‰$.

[2]The relationship between $\delta^{15}N$ and EPA follows the linear model: EPA (%RBC fatty acid) $=1.04 \cdot \delta^{15}N - 6.7‰$, as previously described for this population [18].

Table 3.7.3: *SCD*, *SLC2A4*, and *SREBF1* polymorphisms with MAF ≥0.05

Gene[1]	Chr[2]	SNP[3]	Allele[4]	MAF[5]	Genotype[6]			Individuals[7] Genotyped	HWE p-value[8]
SCD	10	rs1502593	G>A	0.21	747	266	26	1039	0.361
SCD	10	rs522951	C>G	0.34	482	470	113	1065	0.600
SCD	10	rs11190480	A>G	0.12	871	241	16	1128	0.689
SCD	10	rs3071	A>C	0.19	764	271	17	1052	1.000
SCD	10	rs3829160	G>A	0.34	487	473	110	1070	0.727
SCD	10	rs2234970	A>C	0.34	487	461	111	1059	0.375
SCD	10	rs599961	T>G	0.32	394	512	166	1072	1.000
SCD	10	rs41290540	A>C	0.12	820	255	1	1076	0.372
SCD	10	rs3978768	A>G	0.33	496	433	113	1042	0.073
SCD	10	rs11557927	T>G	0.09	769	146	7	922	0.195
SCD	10	rs7849	T>C	0.44	279	557	238	1074	0.741
SCD	10	rs2167444	T>A	0.13	799	232	17	1048	0.390
SLC2A4	17	rs2654185	A>C	0.31	615	386	71	1072	0.707
SLC2A4	17	rs5415	T>C	0.5	309	479	215	1003	0.613
SLC2A4	17	rs5417	C>A	0.31	616	382	69	1067	0.702
SLC2A4	17	rs16956647	C>T	0.15	702	326	44	1072	0.252
SLC2A4	17	rs5435	T>C	0.48	310	555	209	1074	0.873
SLC2A4	17	rs3744405	G>A	0.3	621	389	72	1082	0.460
SREBF1	17	rs2297508	G>C	0.46	294	498	212	1004	0.098
SREBF1	17	rs2282180	G>A	0.05	963	107	2	1072	1.000
SREBF1	17	rs9899634	T>A	0.49	266	554	245	1065	0.200
SREBF1	17	rs8066560	T>A	0.44	320	554	197	1071	0.333
SREBF1	17	rs9902941	C>T	0.49	265	561	246	1072	0.340

[1] Candidate gene; [2] Chromosome; [3] Seattle SNPs Genome Variation Server on March 2008 (dbSNP build 126) Version 5.01; [4] Major/ Minor allele; [5] MAF computed using FREQ in S.A.G.E.; [6] AA=Homozygous Recessive, AB= Heterozygous, BB=Homozygous Dominant; [7] Number of individuals genotyped for each SNP; [8] HWE p-values computed using PLINK.

Table 3.7.4: *SCD*, *SLC2A4*, and *SREBF1* polymorphisms associated with fasting lipid phenotypes[1,2]

Gene/ SNP	Chol	HDL	ApoA1	LDL	VLDL	TG
SCD	0.928	0.168	0.774	0.569	0.424	0.227
rs1502593	(β= 0.2 ± S.E.= 0.2)	(β= -0.4 ± S.E.= 0.3)	(β= -0.1 ± S.E.= 0.3)	(β= 0.2 ± S.E.= 0.3)	(β= 0.3 ± S.E.= 0.3)	(β= 0.4 ± S.E.= 0.3)
SCD	0.535	0.544	0.023	0.571	0.480	0.546
rs522951	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)
SCD	0.417	0.029	**0.006**	0.860	0.106	0.113
rs11190480	(β= 0.2 ± S.E.=0.2)	(β= 0.7 ± S.E.=0.3)	**(β= 1.0 ± S.E.=0.4)**	(β= 0.1 ± S.E.=0.3)	(β= -0.6 ± S.E.=0.4)	(β= -0.5 ± S.E.=0.3)
SCD	0.669	0.385	0.867	0.317	0.549	0.374
rs3071	(β= 0.1 ± S.E.= 0.2)	(β= -0.2 ± S.E.= 0.3)	(β= -0.1 ± S.E.= 0.3)	(β= 0.3 ± S.E.= 0.3)	(β= 0.2 ± S.E.= 0.3)	(β= 0.3 ± S.E.= 0.3)
SCD	0.484	0.531	0.021	0.510	0.589	0.659
rs3829160	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.3)	(β= -0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.3)	(β= 0.1 ± S.E.= 0.2)
SCD	0.556	0.492	0.022	0.554	0.376	0.444
rs2234970	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.3)	(β= -0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.3)
SCD	0.938	0.540	0.433	0.774	0.648	0.522
rs599961	(β= 0.0 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)
SCD	0.864	0.762	0.052	0.882	0.464	0.531
rs3978768	(β= 0.0 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= -0.5 ± S.E.= 0.3)	(β= 0.0 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)
SCD	0.515	0.045	0.008	0.965	0.103	0.096
rs11557927	(β= 0.2 ± S.E.= 0.3)	(β= 0.7 ± S.E.= 0.4)	(β= 1.2 ± S.E.= 0.4)	(β= -0.1 ± S.E.= 0.4)	(β= -0.7 ± S.E.= 0.5)	(β= -0.7 ± S.E.= 0.4)
SCD	0.648	0.048	0.008	0.934	0.170	0.092
rs7849	(β= 0.0 ± S.E.= 0.2)	(β= -0.4 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.3 ± S.E.= 0.2)	(β= 0.4 ± S.E.= 0.2)
SCD	0.441	0.059	0.004	0.768	0.168	0.130
rs2167444	(β=0.2 ± S.E.=0.2)	(β= 0.6 ± S.E.=0.3)	**(β= 1.0 ± S.E.=0.4)**	(β= 0.1 ± S.E.=0.3)	(β= -0.5 ± S.E.=0.4)	(β= -0.5 ± S.E.=0.3)
SLC2A4	0.531	0.016	0.166	0.731	0.177	0.148
rs2654185	(β= -0.1 ± S.E.= 0.2)	(β= -0.5 ± S.E.= 0.2)	(β= -0.4 ± S.E.= 0.3)	(β= 0.1 ± S.E.= 0.2)	(β= 0.4 ± S.E.= 0.3)	(β= 0.4 ± S.E.= 0.3)
SLC2A4	0.064	0.001	0.005	0.337	0.054	0.074
rs5415	(β= -0.3 ± S.E.=0.2)	**(β= -0.7 ± S.E.=0.2)**	**(β= -0.7 ± S.E.=0.2)**	(β= -0.2 ± S.E.=0.2)	(β= 0.5 ± S.E.=0.2)	(β= 0.4 ± S.E.=0.2)
SLC2A4	0.336	0.009	0.138	0.917	0.211	0.205
rs5417	(β= -0.2 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.2)	(β= -0.4 ± S.E.= 0.3)	(β= 0.0 ± S.E.= 0.2)	(β= 0.3 ± S.E.= 0.3)	(β= 0.3 ± S.E.= 0.3)
SLC2A4	0.117	0.134	0.060	0.110	0.177	0.308
rs16956647	(β= -0.4 ± S.E.= 0.2)	(β= -0.4 ± S.E.= 0.3)	(β= -0.6 ± S.E.= 0.3)	(β= -0.4 ± S.E.= 0.3)	(β= 0.4 ± S.E.= 0.3)	(β= 0.3 ± S.E.= 0.3)
SLC2A4	0.127	0.003	0.003	0.703	0.124	0.228
rs5435	(β= -0.3 ± S.E.=0.2)	**(β= -0.6 ± S.E.=0.2)**	**(β= -0.7 ± S.E.=0.2)**	(β= -0.1 ± S.E.=0.2)	(β= 0.4 ± S.E.=0.2)	(β= 0.3 ± S.E.=0.2)
SLC2A4	0.226	0.009	0.076	0.933	0.344	0.435
rs3744405	(β= -0.2 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.2)	(β= -0.5 ± S.E.= 0.3)	(β= 0.0 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.3)	(β= 0.2 ± S.E.= 0.3)
SREBF1	0.506	0.824	0.613	0.346	0.894	0.339
rs4925114	(β= -0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= -0.2 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)
SREBF1	0.063	0.554	0.569	0.029	0.490	0.995
rs2297508	(β= 0.3 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.5 ± S.E.= 0.2)	(β= -0.2 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)
SREBF1	0.117	0.272	0.841	0.023	0.979	0.972
rs2282180	(β= -0.5 ± S.E.= 0.4)	(β= 0.5 ± S.E.= 0.5)	(β= 0.1 ± S.E.= 0.5)	(β= 1.0 ± S.E.= 0.5)	(β= 0.0 ± S.E.= 0.5)	(β= 0.0 ± S.E.= 0.5)
SREBF1	0.210	0.285	0.546	0.236	0.586	0.937
rs989963	(β= -0.2 ± S.E.= 0.2)	(β= -0.2 ± S.E.= 0.2)	(β= -0.2 ± S.E.= 0.2)	(β= -0.3 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)
SREBF1	0.025	0.546	0.853	0.014	0.910	0.659
rs8066560	(β= 0.4 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.5 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)
SREBF1	0.220	0.250	0.647	0.237	0.545	0.945
rs9902941	(β= -0.3 ± S.E.= 0.2)	(β= -0.3 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.3 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)

[1]1Association of SNPs in a linear regression model adjusted for age, sex, community membership, lipid medication and n-3 PUFA intake. Multiple test correction for *SCD* (7 tests), *SLC2A4* (6 test), and *SREBF1* (4) were estimated using the spectral decomposition of LD matrix [41]. Results in bold are significant for *SCD* at $p \leq 0.007$, significant for *SCL2A4* at $p \leq 0.008$ and significant for *SREBF1* at $p \leq 0.013$. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

Table 3.7.5: *SCD*, *SLC2A4*, and *SREBF1* polymorphisms associated with obesity phenotypes[1,2]

Gene/ SNP	BMI	PBF	HC	ThC	WC
SCD	0.035	0.098	0.028	0.096	0.142
rs1502593	(β= -0.5 ± S.E.= 0.2)	(β= -0.4 ± S.E.= 0.2)	(β= -0.5 ± S.E.= 0.2)	(β= -0.5 ± S.E.= 0.3)	(β= -0.5 ± S.E.= 0.3)
SCD	0.242	0.786	0.367	0.482	0.397
rs522951	(β= 0.2 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)
SCD	0.143	0.102	0.101	0.378	0.092
rs11190480	(β= -0.4 ± S.E.=0.3)	(β= -0.4 ± S.E.=0.2)	(β= -0.4 ± S.E.=0.3)	(β= -0.3 ± S.E.=0.3)	(β= -0.6 ± S.E.=0.3)
SCD	0.043	0.150	0.028	0.062	0.182
rs3071	(β= -0.5 ± S.E.= 0.3)	(β= -0.3 ± S.E.= 0.2)	(β= -0.6 ± S.E.= 0.3)	(β= -0.6 ± S.E.= 0.3)	(β= -0.4 ± S.E.= 0.3)
SCD	0.306	0.894	0.450	0.627	0.525
rs3829160	(β= 0.2 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)
SCD	0.271	0.848	0.509	0.641	0.444
rs2234970	(β= 0.2 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)
SCD	0.523	0.235	0.347	0.608	0.469
rs599961	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)
SCD	0.265	0.735	0.474	0.516	0.458
rs3978768	(β= 0.2 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)
SCD	0.801	0.494	0.613	0.775	0.612
rs11557927	(β= 0.0 ± S.E.= 0.3)	(β= -0.2 ± S.E.= 0.3)	(β= -0.2 ± S.E.= 0.3)	(β= 0.1 ± S.E.= 0.4)	(β= -0.2 ± S.E.= 0.5)
SCD	0.786	0.492	0.543	0.631	0.866
rs7849	(β= 0.0 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= ± S.E.=)	(β= -0.1 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)
SCD	0.652	0.615	0.760	0.910	0.499
rs2167444	(β= -0.1 ± S.E.=0.3)	(β= -0.1 ± S.E.=0.2)	(β= -0.1 ± S.E.=0.3)	(β= 0.0 ± S.E.=0.3)	(β= -0.2 ± S.E.=0.3)
SLC2A4	0.251	0.117	0.011	0.012	0.456
rs2654185	(β= 0.2 ± S.E.= 0.2)	(β= 0.3 ± S.E.= 0.2)	(β= 0.5 ± S.E.= 0.2)	(β= 0.6 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.3)
SLC2A4	0.273	0.093	**0.005**	0.037	0.235
rs5415	(β= 0.2 ± S.E.=0.2)	(β= 0.3 ± S.E.=0.2)	**(β= 0.5 ± S.E.=0.2)**	(β= 0.5 ± S.E.=0.2)	(β= 0.3 ± S.E.=0.2)
SLC2A4	0.322	0.156	0.016	0.020	0.566
rs5417	(β= 0.2 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.5 ± S.E.= 0.2)	(β= 0.4 ± S.E.= 0.4)	(β= 0.1 ± S.E.= 0.3)
SLC2A4	0.736	0.832	0.725	0.703	0.781
rs16956647	(β= -0.1 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.3)	(β= 0.1 ± S.E.= 0.3)
SLC2A4	0.365	0.099	0.027	0.040	0.097
rs5435	(β= 0.2 ± S.E.=0.2)	(β= 0.3 ± S.E.=0.2)	(β= 0.4 ± S.E.=0.2)	(β= 0.5 ± S.E.=0.2)	(β= 0.4 ± S.E.=0.2)
SLC2A4	0.491	0.267	0.055	0.069	0.846
rs3744405	(β= 0.1 ± S.E.= 0.2)	(β= 0.2 ± S.E.= 0.2)	(β= 0.4 ± S.E.= 0.2)	(β= 0.4 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.3)
SREBF1	0.568	0.489	0.677	0.901	0.729
rs4925114	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)
SREBF1	0.384	0.374	0.658	0.692	0.677
rs2297508	(β= -0.2 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)
SREBF1	0.558	0.772	0.848	0.500	0.421
rs2282180	(β= 0.2 ± S.E.= 0.4)	(β= ± S.E.=)	(β= 0.1 ± S.E.= 0.4)	(β= 0.3 ± S.E.= 0.5)	(β= 0.4 ± S.E.= 0.5)
SREBF1	0.503	0.489	0.643	0.837	0.959
rs989963	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)
SREBF1	0.366	0.464	0.703	0.577	0.769
rs8066560	(β= -0.2 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)	(β= -0.1 ± S.E.= 0.2)
SREBF1	0.480	0.444	0.686	0.892	0.953
rs9902941	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.1 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)	(β= 0.0 ± S.E.= 0.2)

[1]1Association of SNPs in a linear regression model adjusted for age, sex, community membership and n-3 PUFA intake. Multiple test correction for *SCD* (7 tests), *SLC2A4* (6 test), and *SREBF1* (4) were estimated using the spectral decomposition of LD matrix [41]. Results in bold are significant for *SCD* at $p \leq 0.007$, significant for *SCL2A4* at $p \leq 0.008$ and significant for *SREBF1* at $p \leq 0.013$. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

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3.9 APPENDIX

Supplementary Table 3.9.1: Sequence information used to design primers for *SLC2A4* SNPs identified through resequencing[1].

SNP	FASTA Sequence
SNP 12	TGGAGGGCAGGGGTGGGGGAAACAGGAAGGGAGCCACTGCTGGGTGCCCTCACCCTCACAG CCTCACTCTGTCTGCCTGCCAGGAAAAGGGCCATGCTGGTCAACAATGTCCTGGCGGTGCTGG GGGGCAGCCTCATGGGCCTGGCCAATGCTGCTGCCTCCTATGAAATGCTCATCCTTGGACGAT TCCTCATTGGCGCCTACTCAGGTACTCACGGGCACCACAGCCCTGCCTAGCGCCCTGTTCTCT TTCACCATGCCTGGGCTTTCAGATGGGAATGGACACCTGCCCTCAGCCCTCTCTTCTTCCCTCG CCCAGGGCTGACATCAGGGCTGGTGCCCATGTACGTGGGGGAGATTGCTCCCACTCACCTGC GGGGCGCCCTGGGGACGCTCAACCAACTGGCCATTGTTATCGGCATTCTGATCGCCCAGGTG ACC **[A/G]** GAGCAAGCCTCATGGGTGCCTGGGCAGTGGTTAGAGTGGGGCTCTGGAGAATATGGTGGGCT TCCAAGGTAAGGCAGAAGGGCTGAGTGACCTGCCTTCTTTCCCAACCTTCTCCCACAGGTGCT GGGCTTGGAGTCCCTCCTGGGCACTGCCAGCCTGTGGCCACTGCTCCTGGGCCTCACAGTGCT ACCTGCCCTCCTGCAGCTGGTCCTGCTGCCCTTCTGTCCCGAGAGCCCCCGCTACCTCTACATC ATCCAGAATCTCGAGGGGCCTGCCAGAAAGAGTAAGCTCTCCCGCTGCAGCCTGGCCCAGGC CCATGCCTCCGCCTCATCTTGCTAGCACCTGGCTTCCTCTCAGGTCCCCTCAGGCCTGACCTTC CCTTCTCCAGGTCTGAAGCG
SNP 13	TCCCCGGGGCAAGTACACCTGGCCCGTCCTCTCCTCTCAGACCCCACTGTCCAGACCCGCAGA GTTTAAGATGCTTCTGCAGCCCGGGATCCTAGCTGGTGGGCGGAGTCCTAACACGTGGGTGG GCGGGGCCTTTTGTTCCAGGGACTCTTTTCTCAAAACTTCCCAGTCGGAGGCTGGCGGGAACC CGAGAGGCGTGTCTCGCCAGCCACGCGGAGGGGCGTGGCCTC **[A/G]** TTGGCCCGCCCCACCAACTCCAGCCAAACTCTAAACCCCAGGCGGAGGGGGCGTGGCCTTCT GGGGTGTGCGGGCTCCTGGCCAATGGGTGCTGTGAAGGGCGTGGCCCGCGGGGGCAGGAGC GAGGTGGCGGGGGCTTCTCGCGTCTTTTCCCCCAGCCCCGCTCCACAAGATCCGCGGGAGCCC CACTGCTCTCCGGGTCCTTGGCTTGTGGCTGTGGGTCCCATCGGGCCCGCCCTCGCACGTCAC TCCGGGACCCCCGCGGCCTCCGCAGGTTCTGCGCTCCAGGCCGGAGTCAGAGACTCCAGGAT CGGTTCTTTCATCTTCGCCGCCCCTGCGCGTCCAGCTCTTCTAAGACGAGATGCCGTCGGGCT TCCAACAGATAGGCTCCGAAGTAGGATTCATCATGAGGGGGCGGGGCGGGGGGGCACGGGT CCCGCTTTTCTTGGGCTGGGGTCGCGGTTGGGGTCAGCTGGGGGTGGTTCCTGCGCAGGCGCA GGGGGTGAAGGTAGGGGGCTGGCTATTTATAC

[1] FASTA sequence for SNP 12 and SNP 13 upstream/ downstream primers extracted from Homo sapiens build 37.3 genome database, accession number: NT_010718.16.

Supplementary Table 3.9.2: Pairwise linkage disequilibrium (r^2) between *SCD* polymorphisms with MAF ≥ 0.05.

	rs1502593	rs522951	rs11190480	rs3071	rs3829160	rs2234970	rs599961	rs3978768	rs11557927	rs7849	rs2167444
rs1502593		1.00	0.99	0.90	1.00	1.00	1.00	1.00	0.98	0.57	0.98
rs522951	1.00		0.99	1.00	0.98	0.97	0.31	0.96	1.00	0.53	0.99
rs11190480	0.99	0.99		0.99	1.00	1.00	0.99	0.99	0.82	0.64	0.95
rs3071	0.90	1.00	0.99		1.00	1.00	1.00	0.99	0.98	0.60	1.00
rs3829160	1.00	0.98	1.00	1.00		0.99	0.31	0.97	1.00	0.53	0.99
rs2234970	1.00	0.97	1.00	1.00	0.99		0.31	0.97	1.00	0.53	1.00
rs599961	1.00	0.31	0.99	1.00	0.31	0.31		0.31	1.00	0.60	1.00
rs3978768	1.00	0.96	0.99	0.99	0.97	0.97	0.31		1.00	0.52	1.00
rs11557927	0.98	1.00	0.82	0.98	1.00	1.00	1.00	1.00		0.74	0.85
rs7849	0.57	0.53	0.64	0.60	0.53	0.53	0.60	0.52	0.74		0.64
rs2167444	0.98	0.99	0.95	1.00	0.99	1.00	1.00	1.00	0.85	0.64	

Pairwise linkage disequilibrium (r^2) was calculated using the hapfreq command in FBAT [34].

Supplementary Table 3.9.3: Pairwise linkage disequilibrium (r^2) between *SLC2A4* polymorphisms with MAF ≥ 0.05.

	rs2654185	rs5415	rs5417	rs16956647	rs5435	rs3744405
rs2654185		0.38	0.99	1.00	0.38	0.93
rs5415	0.38		0.37	0.46	0.74	0.33
rs5417	0.99	0.37		1.00	0.37	0.93
rs16956647	1.00	0.46	1.00		0.43	0.99
rs5435	0.38	0.74	0.37	0.43		0.37
rs3744405	0.93	0.33	0.93	0.99	0.37	

Pairwise linkage disequilibrium (r^2) was calculated using the hapfreq command in FBAT [34].

Supplementary Table 3.9.4: Pairwise linkage disequilibrium (r^2) between *SREBF1* polymorphisms with MAF ≥ 0.05.

	rs4925114	rs2297508	rs2282180	rs9899634	rs8066560	rs9902941
rs4925114		0.55	0.83	0.54	0.55	0.55
rs2297508	0.55		1.00	0.82	0.93	0.83
rs2282180	0.83	1.00		0.85	0.99	0.85
rs9899634	0.54	0.82	0.85		0.78	1.00
rs8066560	0.55	0.93	0.99	0.78		0.78
rs9902941	0.55	0.83	0.85	1.00	0.78	

Pairwise linkage disequilibrium (r^2) was calculated using the hapfreq command in FBAT [34].

Supplementary Table 3.9.5: Interaction of n-3 PUFAs with SNPs associated with fasting lipid phenotypes

Gene	SNP	Chol	HDL	ApoA1	LDL	VLDL	TG
SCD	rs1502593	0.8662	0.3811	0.8268	0.5660	0.2541	0.4402
SCD	rs522951	0.2281	0.8279	0.7315	0.0829	0.4033	0.9184
SCD	rs11190480	**0.0047**	0.9044	0.7741	0.0114	0.2841	0.2141
SCD	rs3071	0.9051	0.6751	0.8079	0.7486	0.2077	0.4208
SCD	rs3829160	0.1895	0.7512	0.6030	0.0435	0.5592	0.9704
SCD	rs2234970	0.1831	0.8314	0.6240	0.0403	0.5529	0.9297
SCD	rs599961	0.2418	0.4961	0.2955	0.4177	0.4964	0.5826
SCD	rs3978768	0.4872	0.5900	0.2671	0.1544	0.5402	0.9584
SCD	rs11557927	0.0367	0.4547	0.2774	0.1164	0.8575	0.8680
SCD	rs7849	0.2757	0.9062	0.9227	0.1966	0.2482	0.7979
SCD	rs2167444	**0.0068**	0.3715	0.2477	0.0420	0.5656	0.4656
SLC2A4	rs2654185	0.2275	0.7318	0.8961	0.2581	0.2134	0.4742
SLC2A4	rs5415	0.4927	0.3046	0.8049	0.1734	0.2367	0.3403
SLC2A4	rs5417	0.2320	0.7664	0.8896	0.2443	0.2279	0.4614
SLC2A4	rs16956647	0.4212	0.4561	0.5396	0.6295	0.3108	0.2143
SLC2A4	rs5435	0.1049	0.9924	0.4728	0.0289	0.9975	0.9698
SLC2A4	rs3744405	0.1786	0.5428	0.4536	0.1921	0.4040	0.6017
SREBF1	rs4925114	0.6395	0.7611	0.6492	0.3862	0.7563	0.6924
SREBF1	rs2297508	0.4669	0.3693	0.5635	0.8818	0.5992	0.3834
SREBF1	rs2282180	0.3959	0.6507	0.9422	0.2718	0.2324	0.2260
SREBF1	rs9899634	0.3962	0.4781	0.8808	0.6098	0.9859	0.9324
SREBF1	rs8066560	0.7545	0.3883	0.8447	0.9583	0.7097	0.7330
SREBF1	rs9902941	0.3351	0.4362	0.8801	0.5589	0.9669	0.9511

[1]Interaction of SNPs and n-3 PUFA intake in a linear regression model adjusted for age, sex, community membership and lipid medication. Multiple test correction for *SCD* (7 tests), *SLC2A4* (6 test), and *SREBF1* (4) were estimated using the spectral decomposition of LD matrix [41]. Results in bold are significant for *SCD* at $p \leq 0.007$, significant for *SCL2A4* at $p \leq 0.008$ and significant for *SREBF1* at $p \leq 0.013$.

[2]Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

Supplementary Table 3.9.6: Interaction of n-3 PUFAs with *SCD* SNPs associated with cholesterol after correction for HDL-Cholesterol

Gene	SNP	HDL
SCD	rs11190480	0.0113
SCD	rs2167444	0.0494

[1]Interaction of SNPs and n-3 PUFA intake in a linear regression model adjusted for age, sex, community membership, lipid medication and HDL-cholesterol. Multiple test correction for *SCD* (7 tests) was estimated using the spectral decomposition of LD matrix [41]. Results in bold are significant for *SCD* at $p \leq 0.007$.

[2]Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

Supplementary Table 3.9.7: Interaction of n-3 PUFAs with SNPs associated with obesity phenotypes

Gene	Chr	SNP	BMI	PBF	HC	ThC	WC
SCD	10	rs1502593	0.1497	0.1159	0.2032	0.4985	0.0906
SCD	10	rs522951	0.5770	0.4491	0.4985	0.2010	0.5257
SCD	10	rs11190480	0.3189	0.4121	0.6211	0.6051	0.1512
SCD	10	rs3071	0.1953	0.1911	0.3325	0.2852	0.0769
SCD	10	rs3829160	0.6801	0.4347	0.5418	0.2593	0.5929
SCD	10	rs2234970	0.6465	0.5018	0.5480	0.2448	0.6016
SCD	10	rs599961	0.3996	0.7416	0.7654	0.5990	0.1629
SCD	10	rs3978768	0.6603	0.4614	0.5171	0.2304	0.5954
SCD	10	rs11557927	0.0208	0.0745	0.0907	0.1139	0.0406
SCD	10	rs7849	0.9300	0.8702	0.9177	0.7431	0.3354
SCD	10	rs2167444	0.2666	0.4612	0.5347	0.5101	0.1921
SLC2A4	17	rs2654185	0.4226	0.7133	0.8995	0.3010	0.9028
SLC2A4	17	rs5415	0.3955	0.4168	0.6639	0.5996	0.3415
SLC2A4	17	rs5417	0.7591	0.6869	0.8890	0.2810	0.8792
SLC2A4	17	rs16956647	0.2915	0.2121	0.5556	0.1387	0.5453
SLC2A4	17	rs5435	0.8464	0.6722	0.7169	0.9704	0.8436
SLC2A4	17	rs3744405	0.2330	0.4205	0.2421	0.1065	0.3141
SREBF1	17	rs4925114	0.9581	0.6534	0.8754	0.3503	0.8454
SREBF1	17	rs2297508	0.4590	0.3597	0.4123	0.5249	0.4195
SREBF1	17	rs2282180	0.7614	0.1722	0.8973	0.5014	0.6044
SREBF1	17	rs9899634	0.4897	0.3004	0.5648	0.2227	0.3570
SREBF1	17	rs8066560	0.7541	0.4570	0.6558	0.7643	0.6912
SREBF1	17	rs9902941	0.3957	0.2636	0.4799	0.1768	0.3059

[1]Interaction of SNPs and n-3 PUFA intake in a linear regression model adjusted for age, sex, community membership and lipid medication. Multiple test correction for *SCD* (7 tests), *SLC2A4* (6 test), and *SREBF1* (4) were estimated using the spectral decomposition of LD matrix [41]. Results in bold are significant for *SCD* at $p \leq 0.007$, significant for *SCL2A4* at $p \leq 0.008$ and significant for *SREBF1* at $p \leq 0.013$.

[2]Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

OBESITY POLYMORPHISMS IDENTIFIED IN GENOME-WIDE ASSOCIATION STUDIES INTERACT WITH n-3 POLYUNSATURATED FATTY ACID INTAKE AND MODIFY GENETIC ASSOCIATIONS WITH ADIPOSITY PHENOTYPES IN YUP'IK PEOPLE[3]

4.1 ABSTRACT

BACKGROUND: n-3 polyunsaturated fatty acids (n-3 PUFAs) have anti-obesity effects that may modulate risk of obesity, in part, through interactions with genetic factors. Genome-wide association studies (GWAS) have identified genetic variants associated with BMI; however the extent to which these variants influence adiposity through interactions with n-3 PUFAs remains unknown. DESIGN: We evaluated 10 obesity GWAS SNPs for individual and cumulative associations with adiposity phenotypes in a cross-sectional sample of Yup'ik people (n=1073) and evaluated whether genetic associations with obesity were modulated by n-3 PUFA intake. A genetic risk score (GRS) was calculated by adding the BMI-increasing alleles across all 10 SNPs. Dietary intake of n-3 PUFAs was estimated using nitrogen stable isotope ratios ($\delta^{15}N$) of red blood cells (RBC) and genotype-phenotype analyses were tested in linear models accounting for familial correlations. RESULTS: *FTO* was positively associated with percent body fat (PBF, p=0.0039) and *ETV5* was negatively associated with hip and thigh circumference (HC, p=0.002 and ThC, p=0.003, respectively). GRS was positively associated with adiposity phenotypes including: BMI (p=0.012), PBF (p=0.022), ThC (p=0.025) and waist circumference (WC, p=0.038). The variance in adiposity phenotypes explained by the GRS included: BMI (0.7%), PBF (0.3%), ThC (0.7%) and WC (0.5%). GRS interactions with n-3 PUFAs modified the association with adiposity

[3] Lemas, DJ, Klimentidis YC, Wiener HW, O'Brien DM, Hopkins S, Allison DB, Fernandez, JR, Tiwari HK, Boyer BB. 2012. Obesity Polymorphisms Identified in Genome-Wide Association Studies Interact with n-3 Polyunsaturated Fatty Acid Intake and Modify Genetic Associations with Adiposity Phenotypes in Yup'ik People. Prepared for submission to *The International Journal of Obesity*.

and accounted for more than twice the phenotypic variation (~1-2%), relative to GRS associations alone. CONCLUSION: Obesity GWAS SNPs contribute to adiposity in this study population and interactions with n-3 PUFA intake potentiated the risk of fat accumulation among individuals with high obesity GRS. These data suggest the anti-obesity effects of n-3 PUFAs among Yup'ik people, in part, may be dependent upon an individual's genetic predisposition to obesity.

4.2 INTRODUCTION

Obesity contributes to a series of metabolic abnormalities including elevated triglycerides and inflammatory cytokines, and insulin resistance which ultimately increases the risk of developing type 2 diabetes (T2D) and other diseases.[1] Given the increasing public health burden associated with obesity and the difficulty achieving and maintaining weight loss,[2-4] it is important to define mechanisms that influence body weight.

The heritability of obesity has been estimated to between 40-70%.[5,6] A recent meta-analysis of obesity genome-wide association studies (GWAS) conducted primarily in European samples has identified single nucleotide polymorphisms (SNPs) associated with variation in BMI.[7] The extent to which these loci are associated with adiposity phenotypes in other ethnic groups is unclear.[8,9] Furthermore, although considerable progress has been made in elucidating SNPs associated with excess body weight, the heritability of obesity explained by these loci remains low (<2%).[7] Increasingly, gene-by-environment interactions have been suggested to account for the "missing heritability" in complex traits.[10] Experimental evidence demonstrating that interactions between genetic variants and environmental factors partially account for the heritability of obesity may help elucidate important mechanisms that can modulate the risk of obesity related co-morbidities through lifestyle interventions.

Total dietary fat has been implicated in the development of obesity,[11] however a growing body of evidence indicates that not all dietary fatty acids are equally

obesigenic.[12] Regular consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) may reduce adiposity in humans,[13] in part, by inhibiting adipogenesis[14] and stimulating fat oxidation.[15] Animal models have shown significant reductions in fat mass when dietary n-3 PUFAs, namely eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), are substituted for saturated fats,[16] monosaturated fats,[17] and n-6 polyunsaturated fats,[18] after controlling for caloric intake. These findings have largely been confirmed by human studies,[19] however information regarding whether the anti-obesity effects attributed to n-3 PUFA is dependent upon a specific genotype remains limited.[20] Knowledge about the interplay between genetic factors and consumption of n-3 PUFA may facilitate the choice of more effective and specific measures of obesity prevention based upon individualized genetic make-up.

Given the anti-obesigenic effects of n-3 PUFAs, the widely varying intake of n-3 PUFA in this study population of Yup'ik people (nearly 30-fold range), and the availability of a precise biomarker for n-3 PUFA intake ($\delta^{15}N$; [21]); we evaluated whether n-3 PUFA intake modifies the association between GWAS SNPs and adiposity phenotypes. The individual and cumulative effects of 10 SNPs reproducibly associated with BMI[22–26] were tested in a sample of Yup'ik people from the Center for Alaska Native Health Research (CANHR) study. We evaluated the cumulative effects of these SNPs using a genetic risk score (GRS) that estimates an individual's genetic predisposition to obesity by adding the BMI-increasing alleles across all 10 SNPs.

4.3 METHODS

4.3.1 PARTICIPANTS AND STUDY DESIGN

The CANHR studies genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik people.[27] Recruitment of Yup'ik participants was initiated in 2003 and continues in 11 Southwest Alaskan communities. All residents ≥14 years old were invited to participate and the resulting distribution of age in our study sample reflects the age distribution

among eligible participants according to 2000 U.S. census data. Participants signed informed-consent documents before entering the study using protocols that were approved by the University of Alaska Institutional Review Board, the National and Alaska Area Indian Health Service Institutional Review Boards, and the Yukon Kuskokwim Human Studies Committee. Summary statistics regarding family data were calculated using PEDINFO in the Statistical Analysis for Genetic Epidemiology (S.A.G.E., 2009) software. The analyses in this report included 1073 non-pregnant Yup'ik participants (41 founders, 920 non-founders, and 112 singletons) with ages ranging between 14 and 94 years at enrollment. There were 195 pedigrees in this data set with a mean size 5.52 individuals (range, 1-849) and 696 sibships with a mean size of 1.32 (range, 1-9).

4.3.2 ANTHROPOMETERIC AND BIOCHEMICAL MEASUREMENT

Trained staff obtained anthropometric measurements using protocols from the NHANES III Anthropometric Procedures Manual [28] as previously described.[29] These measurements included height, weight and 4 circumferences (waist, hip, triceps, and thigh). Percent body fat (PBF) was measured by electrical bioimpedance using a Tanita TBF-300A bioimpedance analyzer (Tanita Corp, Arlington Heights, IL, U.S.A.).

4.3.3 BIOMARKER OF n-3 PUFA INTAKE

n-3 PUFA intake was assessed using the nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells (RBC), which has been validated as a biomarker for EPA and DHA intake as previously described.[21] The time to 50% turnover of RBC is approximately 45 days [30], therefore, the mean RBC $\delta^{15}N$ values reflect a mean n-3 PUFA intake over 1.5 months. 1.8 µl aliquots of RBC were pipetted into 3.5 x 3.75 mm tin capsules, autoclaved for 20 minutes at 121°C to destroy blood-borne pathogens, and dried to a constant mass of 0.2 - 0.4 mg. Capsules were crushed into a ball for loading into an autosampler. Samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio mass

spectrometry, using a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo-Finnigan Inc., Breman, Germany). Isotope ratios are analyzed relative to IAEA-certified reference materials calibrated to atmospheric nitrogen, for which $^{15}N/^{14}N = 0.0036765$. By convention and for ease of interpretation, isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \cdot 1000‰$. We concurrently prepared and ran multiple laboratory standards (peptone, $\delta^{15}N = 7.00$) to assess analytical accuracy and precision; these were analyzed after every eighth sample and gave values of $\delta^{15}N = 7.01 \pm 0.24‰$ (mean ± SD). The range of isotopic variation in our dataset (9‰) was very large relative to analytical precision (0.2‰). We modeled the effects of n-3 PUFA intake as a categorical variable with four groups by quartile, which is hereafter referred to as $\delta^{15}N$.

4.3.4 SNP SELECTION AND GENOTYPING

SNP genotyping for the present study was completed in 2009 and include 10 obesity loci that have recently been implicated in an obesity GWAS meta-analysis.[7] In situations where more than a single SNP has been identified for a given gene, we genotyped the SNP most frequently reported in these studies. Genotyping was carried out at the Broad Institute (Cambridge, MA) by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight spectrometry on a Sequenom iPLEX platform.[31] We compared the frequency of the GWAS BMI-increasing 'risk alleles' in Yup'ik people to five HapMap populations.[32]

4.3.5 QUALITY CONTROL OF PHENOTYPIC AND GENOTYPIC DATA

Simple linear models were fit to each phenotype using all covariates (age, sex, community group) included in the association models, and the distributions of the

residuals for each phenotype were examined for normality with the R statistical programming language (v2.10.1, R Development Core, 2009). We considered a series of transformations (square root, log, inverse) to improve normality and the Box-Cox transformation [33] was identified as the best procedure for phenotypes whose residuals did not follow a normal distribution. Information regarding the power transformation for each adiposity phenotypes is presented in **Supplementary Table 4.9.1**. Family data were stored in the Progeny database (Progeny Software LLC, South Bend, IN, U.S.A.) and merged into a single extended pedigree comprised of multiple independent families using PedMerge.[34] Genotypic data were tested for Mendelian inconsistencies using PEDCHECK.[35] In this sample, Illumina IV linkage panel (Illumina, Inc., San Diego, CA, USA) genotypes were available from an ongoing linkage study and were used to construct principal components using the EIGENSTRAT analysis package.[36] The first PC did not have an obvious interpretation to the data however the second PC discriminated individuals into two groups that corresponded to either coastal or inland communities. Based on this, we defined a dichotomous community group variable that indicates either coastal or inland. We assessed Hardy-Weinberg equilibrium (HWE) in the founders using PLINK (v1.07)[37] and determined minor allele frequency (MAF) for each SNP using the FREQ module in the Statistical Analysis for Genetic Epidemiology (S.A.G.E 2009) program. The present study restricted association and interaction analysis to SNPs with MAF $\geq$ 5% that did not deviate from HWE ($p \leq 0.005$). HWE multiple test correction was determined using a Bonferroni correction for 10 tests ($\alpha \leq 0.005$).

4.3.6 ASSOCIATION ANALYSIS

Each SNP was tested using as additive model for association with obesity-related phenotypes using the ASSOC module in the S.A.G.E. software package.[38] We included both demographic (age, community group, and sex) and environmental covariates ($\delta^{15}N$) in the association analysis and calculated likelihood ratio statistics to compare 4 nested models. Model 1 included demographic covariates (age, sex and community group);

Model 2 included baseline covariates and SNP to test for an additive genetic effect of SNP (defined as the number of minor alleles); Model 3 included demographic covariates, the additive genetic effect of SNP, and $\delta^{15}N$ (defined by quartiles of $\delta^{15}N$) as an estimate of n-3 PUFA intake; Model 4 included all covariates from Model 3 and an interactions between the additive genetic effect and $\delta^{15}N$ categories. Note that the model 4 is the only model to test gene-diet interactions. Correction for multiple testing in genetic analyses (models 1-4) for SNPs with MAF $\geq$0.05 employed a Bonferroni correction for 10 tests ($\alpha \leq 0.005$). Effect sizes (β) were presented for association analyses as the change in transformed phenotypes according to BMI-increasing "risk allele" adjusted for demographic and environmental covariates.

4.3.7 GENETIC RISK SCORE ANALYSIS

The obesity genetic risk score (GRS) was calculated by summing the number of risk alleles that each individual possessed. The obesity risk alleles were defined as those that were associated with higher BMI (defined as BMI $\geq$ 25 kg/m^2) in previous GWAS studies.[7,25,26] To account for missing genotypes, we divided the sum of risk alleles by the number of loci included in the score. The GRS was split into three groups by tertiles [39] and a categorical variable was created to allow for non-linear effects without being unduly influenced by extreme values. We tested the GRS for association with obesity traits using two models (model 5 and 6) run with the ASSOC module in S.A.G.E. Model 5 included the GRS as a main effect and controlled for demographic (sex, age, and community group) covariates. Model 6 tested the GRS for interaction with $\delta^{15}N$ and controlled for demographic covariates and $\delta^{15}N$. Model 7 tested for interactions ($\delta^{15}N$*GRS) in a model that included demographic covariates and interactions between the GRS and quartiles of $\delta^{15}N$. Note that model 7 is the only model to test gene-diet interaction. In a subset of participants (n=488), we evaluated 24 Hour Recalls (24HR) to estimate total energy (kcal) and total fat intake (% calories) as potential confounding dietary variables in the $\delta^{15}N$*GRS interaction model (model 7). The variance explained

by the fixed effects in the GRS association models were calculated as the difference between the total variance of the trait and the sum of the random effects in the model (i.e. the residual variance and the family effect). The proportion of variance explained by random effects was calculated whereby the ratio of the difference noted above was included in the numerator and the total variance of the trait was included in the denominator. We defined the proportion of the variance explained by each term in the GRS association model as the difference in proportion of variance explained by models with and without the terms of interest. Results were considered significant in the GRS analyses (models 5-7) if the p-value was ≤ 0.05 (2-tailed).

4.4 RESULTS

4.4.1 DESCRIPTIVE STATISTICS

Descriptive statistics on Yup'ik men (n=510) and women (n=563) enrolled in this study are presented in **Table 4.7.1**. Yup'ik women in this study had a mean age of 38.5 (±1.6) years and had more adiposity than men that reported a mean age of 36.6 (±0.6) years. Specifically, women had more adiposity that included greater BMI ($p<0.0001$), percentage body fat ($p<0.0001$) and hip circumference ($p<0.0001$) compared to men. Interestingly, we did not detect significant differences between women and men with respect to waist circumference.

4.4.2 DISTRIBUTION OF $\delta^{15}N$ IN THE STUDY POPULATION

We assessed n-3 PUFA intake in 1073 Yup'ik participants using RBC $\delta^{15}N$ as a biomarker of EPA and DHA intake. Summary statistics are grouped by gender and $\delta^{15}N$ quartiles and reported in **Table 4.7.2**. The mean $\delta^{15}N$ value in this study was 9.0‰, and ranged from 6.4‰ to15.2‰. According to the linear relationship between RBC $\delta^{15}N$ and RBC EPA described elsewhere for this study population,[21] the corresponding mean EPA (%RBC fatty acids) was 2.66%. Measurement of $\delta^{15}N$ by gender yielded means of 9.1‰ for females and 8.7‰ for males. The mean RBC $\delta^{15}N$ values according to the four

groups by quartile were: 7.3‰, 8.2‰, 9.1‰, 11.0‰ in groups 1-4, respectively. These values correspond to EPA (% RBC fatty acids) group means of: 0.9%, 1.8%, 2.8%, and 4.7% [21]. The standard deviation of $\delta^{15}N$ in this sample did not differ according to gender (1.5‰ for both females and males).

4.4.2 GENETIC VARIATION AMONG OBESITY SNPS

Allele frequencies for 10 SNPs that showed significant association with obesity and/or BMI in large-scale GWAS are presented in **Table 4.7.3**. The frequencies for risk alleles observed in this sample of Yup'ik people were comparable to frequencies reported in other populations (**Table 4.7.3**). Notable exceptions include the rs10838738 (*MTCH2*) and the rs7498665 (*SH2B1*) risk alleles which are at higher frequencies among this sample of Yup'ik people. All genotyped SNPs were consistent with expected HWE ($p>0.002$) and had MAF $\geq$ 0.05 with the exception of rs6265 (*BDNF*) which had MAF=0.04.

4.4.3 INDIVIDUAL SNPS AND ADIPOSITY PHENOTYPES

Without adjustment for n-3 PUFA intake ($\delta^{15}N$), no SNPS were significantly associated with adiposity phenotypes after Bonferroni multiple test correction (**Supplementary Table 4.9.2**). Although not significant, 8 of the 10 SNPs tested in this study had direction-consistent associations trends with BMI that were in agreement with reports from GWAS. After adjusting our analyses for n-3 PUFA intake ($\delta^{15}N$), we found that rs9939609 (*FTO*) and rs7647305 (*ETV5*) risk alleles were associated with adiposity phenotypes (**Figure 4.6.1** and **Table 4.7.4**). Specifically, rs9939609 (*FTO*) was positively associated with percent body fat (PBF, p=0.004) and rs7647305 (*ETV5*) was negatively associated with hip and thigh circumference (HC, p=0.002 and ThC, p=0.003, respectively). We did not detect significant SNP interactions with n-3 PUFA intake that modified any associations with adiposity phenotypes (**Supplementary Table 4.9.3**).

4.4.4 GENETIC RISK SCORE AND ADIPOSITY PHENOTYPES

The GRS was positively associated with adiposity as measured by BMI (p=0.012), PBF (p=0.022), ThC (p=0.025) and WC (p=0.038) after adjusting for n-3 PUFA intake ($\delta^{15}N$) (**Table 4.7.5**). The GRS interactions with n-3 PUFA intake were significant for BMI (p=0.011), PBF (p=0.025), and WC (p=0.018). **Figure 4.6.2** and **Table 4.7.5** show that n-3 PUFA intake significantly modifies the GRS association with BMI, such that people with the highest BMI also had high levels n-3 PUFA intake (Q4) and a high GRS (T3). In contrast, our results show that people with a low GRS (T1) and high levels of n-3 PUFA intake (Q4) had a lower BMI, relative to individuals with the same GRS (T1) and low n-3 PUFA (Q1) intake (**Figure 4.6.1**). The GRS interaction with n-3 PUFA explained approximately twice the phenotypic variation in adiposity phenotypes relative to GRS associations with adiposity alone (**Table 4.7.6**).

4.5 DISCUSSION

In this study, we evaluated 10 SNPs identified in a previous meta-analysis of obesity GWAS[7] for individual and cumulative associations with adiposity phenotypes in a cohort of Yup'ik people and assessed whether exposure to n-3 PUFAs modified these associations. After corrections for n-3 PUFA consumption, we found rs9939609 (*FTO*) was positively associated PBF and rs7647305 (*ETV5*) was negatively associated with ThC and HC. Interestingly, controlled feeding studies in rodents have shown that n-3 PUFAs decrease adiposity by reducing central fat mass;[40] however epidemiological evidence in humans supporting the anti-obesity effects of n-3 PUFAs remains controversial.[13] Our analyses indicate that genetic predisposition to obesity, as measured by the GRS, was positively associated with adiposity and interactions with n-3 PUFA intake modified these associations whereby among individuals with a low GRS, n-3 PUFA intake did not increase BMI. However, if you have a moderate (2) or high (3) GRS, n-3 PUFAs intake is obesigenic. Taken together, these results suggest the anti-obesity effects of n-3 PUFA in humans may be dependent upon an individual's genetic

predisposition to obesity. Consistently, GWAS have identified SNPs associated with BMI in European populations that are located within the first intron of *FTO*.[7,22,26] To date, most *FTO* candidate gene studies have been conducted on the rs9939609 SNP mainly because this variant has the strongest known association of any SNP with BMI.[41] Subsequent to the discovery of the rs9939609 SNP, European,[42] African,[43] and Asian[44] study populations have largely confirmed the association of *FTO* with BMI, although this is not without exception.[9,45] Our data indicates the rs9939609 (*FTO*) risk allele was positively associated with PBF in Yup'ik people and supports other studies indicating that variation in *FTO* contributes to fat accumulation.[46]

In contrast to rs9939609 (*FTO*), candidate gene studies that have replicated the rs7647305 (*ETV5*) association with obesity remain limited.[47–49] A prospective sample of 7,146 European children showed the *ETV5* (rs7647305) SNP was associated with increased BMI and body weight [47] and a cohort of 18,014 middle-aged Danish adults demonstrated the *ETV5* (rs7647305) SNP was associated with increased odds of being overweight and/or obese.[49] Finally a prospective study among 20,428 European adults with an average follow-up of 12.9 years demonstrated the *ETV5* (rs7647305) SNP was associated with protection from developing T2D and this association was stronger after correcting for BMI.[48] Our study was not able to evaluate whether the *ETV5* (rs7647305) SNP was associated with protection from developing T2D in Yup'ik people due to our cross-sectional study design. However, our results do support the *ETV5* (rs7647305) locus in obesity pathogenesis by showing the *ETV5* (rs7647305) C allele was associated with reduced HC and ThC and near associations for reduced BMI, PBF, and WC. Moreover, despite the fact that overweight and obesity prevalence among Yup'ik people resembles the prevalence of overweight and obesity in the general U.S. population, T2D prevalence remains low (~3%).[27] These results are consistent with the finding of Li *et al.* (2011) in that ETV5 (rs7647305) was associated with protection from T2D [48]. Interestingly, the *ETV5* (rs7647305) associations with adiposity phenotypes in Yup'ik people were "opposite" to those reported in subjects of Europeans ancestry.[47,49]

Although this lack of consistency can be attributed to type I error, theoretical modeling has argued that "flip-flop" association can be attributed to population differences that occur when the SNP of interest is correlated with the causal variant through linkage disequilibrium.[50]

The GRS, which evaluates the cumulative association of 10 obesity GWAS loci, was positively associated with several measures of obesity (BMI, PBF, ThC, WC) in our study population. Consistent with previous studies, our analysis demonstrated the GRS predicts < 1% of the BMI variation in this sample of Yup'ik people (0.7% for BMI). Li *et al.* included 12 SNPs that explained 0.9% of BMI variation in Europeans,[51] Takeuchi *et al.* calculated a GRS with 14 SNPs that explained 0.65% among Japanese,[52] and Peterson *et al.* included 56 SNPs explained 0.66% of the variation in BMI among a mixed sample of European- and African-Americans.[53] We calculated a GRS in this study using 5 obesity GWAS SNPs that overlap with Li *et al.*, [51] 7 obesity GWAS SNPs that overlap with Takeuchi *et al.*[52] and 9 obesity GWAS SNPs that overlap with Peterson *et al.*.[53] Taken together, these results suggest that while considerable progress has been made in gene discovery, obesity SNPs highly replicated in GWAS do not yet predict a significant proportion of the heritability attributed to adiposity phenotypes.

Increasingly, experimental designs are considering gene-environment interactions to account for the "missing heritability" associated with complex phenotypes like obesity.[10] Given the established contribution of n-3 PUFAs to changes in adiposity phenotypes and the availability of a precise biomarker for n-3 PUFA intake ($\delta^{15}N$) in Yup'ik people,[21] we tested whether individual SNPs and the GRS associations with adiposity phenotypes were modified by consumption of n-3 PUFA. Our analysis demonstrated that elevated n-3 PUFA intake with an increased GRS strengthened the association with adiposity phenotypes. Interestingly, the interaction of GRS with n-3 PUFA intake accounted for approximately twice the phenotypic variation as the genetic risk score alone. These results are consistent with gene-by-environment analysis conducted by Li *et al.* that demonstrated physical activity interactions with a GRS,

calculated from 12 obesity risk alleles, accounted for twice as much phenotypic variation in obesity-related traits relative to the genetic risk score alone.[51]

The strengths of this study include a sample size large enough to detect significant SNP associations and a statistical approach that accounts for relationships among participants while also allowing for covariates. Our study population of Yup'ik people was ideally suited to test the contribution of n-3 PUFA intake [54,55] and genetic factors [56] to changes in adiposity phenotypes. Given the 30-fold range of n-3 PUFA consumption[57] in this study population, an additional strength of this study includes a biomarker of EPA and DHA intake ($\delta^{15}N$) that can be precisely estimated in large epidemiological studies.[21] A potential limitation of our study was that n-3 PUFA intake may be confounded by total fat and total energy intake due to a traditional diet pattern that is high in fat. Given this limitation, it is possible that the $\delta^{15}N$*GRS interaction with adiposity phenotypes may have been influenced by total fat or total energy intake. We evaluated this limitation in a subset of participants (n=488) by including total energy (kcal) and total fat intake (% calories), derived from 24 Hour Recalls (24HR), as an additional covariate in the $\delta^{15}N$*GRS interaction model (model 7). Our follow-up analysis did not find that including total energy and total fat or intake in the $\delta^{15}N$*GRS interaction model significantly changed the results (**Supplementary Table 4.9.4**). These data indicate the GRS*n-3 PUFA interactions were not appreciably confounded by total fat and total energy intake and suggest that n-3 PUFA intake may modulate the risk associated with obesity susceptibility genes.

In conclusion, cross-sectional studies of this nature have potential to elucidate novel gene-diet interactions that may ultimately help account for some of the "missing heritability" associated with obesity.[58] To the extent that genetic predisposition to obesity is estimated by 10 SNPs reproducibly identified through GWAS, our results suggest that including n-3 PUFA interactions increased the ability to detect the contribution of these SNPs to changes in BMI. In contrast, individuals with no genetic predisposition to obesity, as measured by GRS, had less adiposity in the presence of high

n-3 PUFA intake. Although animal and human studies have reported that consumption of n-3 PUFAs may reduce adiposity,[13] our results indicate the anti-obesity effects of n-3 PUFAs may, in part, be dependent upon an individual's genetic predisposition to obesity. Characterizing gene-diet interactions in with high intakes of n-3 PUFAs may help identify individuals that are likely to benefit from specific dietary interventions. Interestingly, these analyses do suggest anti-obesity effects of n-3 PUFA intake are dependent upon an individual's genotype and may play an essential role in determining the risk associated with obesity susceptibility genes among Yup'ik people. Additional genomic studies will be required to replicate these results in large circumpolar populations with widely varying intake of n-3 PUFA in order to determine the validity and public health implication.

4.6 FIGURES

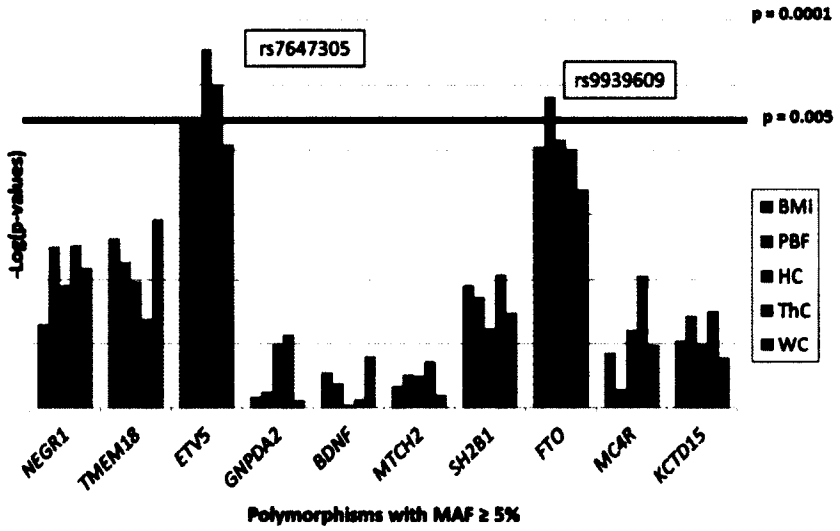

Figure 4.6.5: Obesity GWAS polymorphisms associated with adiposity phenotypes.
Association of SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. The red line represents multiple test correction that was estimated using the Bonferroni correction. Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

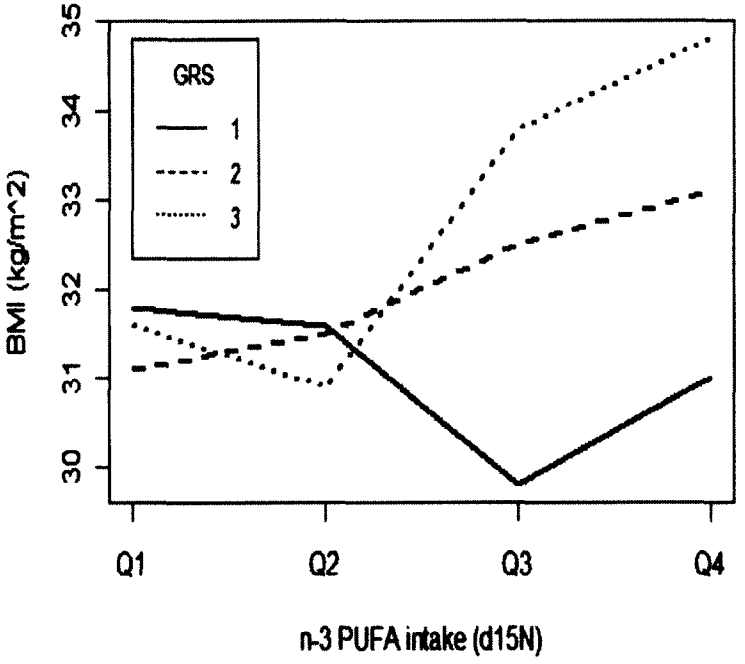

Figure 4.6.6: n-3 PUFA intake modifies the genetic risk score (GRS) association with BMI.
n-3 PUFA intake (δ15N) is represented as a categorical variable with four groups by quartile (Q1-Q4) where Q1 is lowest level of n-3 PUFA intake and Q4 is the highest level of n-3 PUFA intake (p=0.011). The GRS is represented as a categorical variable with three groups by tertiles (T1-T3) where T1 is lowest obesity GRS and T3 is highest obesity GRS.

4.7 TABLES

Table 4.7.1: Descriptive statistics of adiposity phenotypes [1,2]

Variables	Women	Men	P-values
No. of participants	563	510	
Age (yrs)	38.5 ± 1.6	36.6 ± 0.6	0.0740
BMI (kg/m^2)	28.7 ± 5.6	26.3 ± 5.6	<0.0001
Percentage Body Fat (%)	42.8 ± 1.4	27.8 ± 1.1	<0.0001
Waist Circumference (cm)	88.1 ± 21.6	87.5 ± 21.5	0.5039
Hip Circumference (cm)	105.4 ± 43.0	98.7 ± 43.0	<0.0001
Thigh Circumference (cm)	51.1 ± 21.4	50.3 ± 21.3	0.0110

[1] Values are reported as mean (± S.E.) predicted from linear model accounting for familial correlations.

[2] P-values for differences by gender are derived using student t-test.

Table 4.7.2: Distribution of the RBC nitrogen stable isotope ratio ($\delta^{15}N$)[1,2]

		Sex		Quartiles of $\delta^{15}N$**			
	Total	**Women**	**Men**	**Q1**	**Q2**	**Q3**	**Q4**
No. of participants	1073	565	510	262	261	269	281
Mean ± SD (‰)	9.0 ± 1.5	9.1 ± 1.5	8.7 ± 1.5	7.3 ± 0.3	8.2 ± 0.2	9.1 ± 0.3	11.0 ± 1.1
Maximum	15.2	15.2	13.5	7.8	8.6	9.8	15.2
Minimum	6.4	6.4	6.4	6.4	7.8	8.6	9.8
Range (‰)	8.8	8.8	7.1	1.4	0.8	1.2	5.4

[1]Isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(\ ^{15}N/^{14}N_{standard})] \cdot 1000‰$.

[2]The relationship between $\delta^{15}N$ and EPA follows the linear model: EPA (%RBC fatty acid) $=1.04 \cdot \delta^{15}N - 6.7‰$, as previously described for this population.[21]

Table 4.7.3: Obesity GWAS SNP polymorphisms[1,2,3,4]

Gene	SNP[1]	Chr	Risk Allele	Frequency	Frequency of Risk Allele in HAPMAP populations					HWE (p-value)	Reference
					YRI	CEU	HCB	JPT	MEX		
NEGR1	rs2815752	1	A	0.81	0.53	0.64	0.88	0.92	0.73	0.2007	[7,26]
TMEM18	rs7561317	2	G	0.85	0.79	0.85	0.91	0.88	0.88	0.1768	[25]
ETV5	rs7647305	3	C	0.94	0.61	0.79	0.94	0.94	0.81	0.478	[25]
GNPDA2	rs10938397	4	G	0.15	0.21	0.45	0.25	0.37	NA	0.2097	[7,26]
BDNF	rs6265	11	G	0.96	0.99	0.81	0.38	0.63	0.79	1	[7,26]
MTCH2	rs10838738	11	G	0.66	0.04	0.36	0.35	0.35	0.4	0.0037	[26]
SH2B1	rs7498665	16	G	0.66	0.21	0.38	0.15	0.13	0.38	0.462	[25,26]
FTO	rs9939609	16	A	0.19	0.51	0.46	0.12	0.19	NA	0.3223	[22,26]
MC4R	rs17782313	18	C	0.08	0.31	0.27	0.14	0.24	0.14	1	[23,24,26]
KCTD15	rs29941	19	C	0.31	0.86	0.68	0.22	0.26	0.65	0.4179	[7,25]

[1] Seattle SNPs Genome Variation Server on March 2008 (dbSNP build 126) Version 5.01.

[2] Frequency of risk allele computed using FREQ module in S.A.G.E.

[3] Frequency of risk allele among five HapMap populations: Yoruba in Ibadan, Nigeria (YRI), Utah residents with ancestry from northern and western Europe (CEU), Han Chinese in Beijing, China (CHB), Japanese in Tokyo, Japan (JPT), Mexican ancestry in Los Angeles, California.

[4] HWE p-value computed using founders in PLINK.

Table 4.7.4: Obesity polymorphisms associated with adiposity after $\delta^{15}N$ adjustment[1,2]

Gene	SNP	BMI	PBF	HC	ThC	WC
NEGR1	rs2815752	0.222	0.056	0.109	0.055	0.081
		(β= -0.3, SE= 0.2)	(β= -0.4, SE= 0.2)	(β= -0.4, SE= 0.2)	(β= -0.6, SE= 0.3)	(β= -0.5, SE= 0.3)
TMEM18	rs7561317	0.048	0.073	0.103	0.202	0.034
		(β= 0.5, SE= 0.2)	(β= 0.4, SE= 0.2)	(β= 0.4, SE= 0.2)	(β= 0.4, SE= 0.3)	(β= 0.6, SE= 0.3)
ETV5	rs7647305	0.006	0.006	**0.002**	**0.003**	0.009
		(β= -1.5, SE= 0.6)	(β= -1.4, SE= 0.5)	(β= -1.8, SE= 0.6)	(β= -2.0, SE= 0.7)	(β= -1.9, SE= 0.7)
GNPDA2	rs10938397	0.821	0.761	0.315	0.267	0.867
		(β= 0.1, SE= 0.2)	(β= 0.1, SE= 0.2)	(β= 0.2, SE= 0.2)	(β= 0.3, SE= 0.3)	(β= 0.1, SE= 0.3)
BDNF	rs6265	0.531	0.646	0.943	0.859	0.396
		(β= 0.4, SE= 0.6)	(β= 0.2, SE= 0.5)	(β= 0.0, SE= 0.6)	(β= 0.1, SE= 0.7)	(β= 0.6, SE= 0.7)
MTCH2	rs10838738	0.682	0.550	0.556	0.440	0.797
		(β= 0.1 SE= 0.2)	(β= 0.1, SE= 0.2)	(β= 0.1, SE= 0.2)	(β= 0.2, SE= 0.2)	(β= 0.1, SE= 0.2)
SH2B1	rs7498665	0.111	0.136	0.239	0.092	0.179
		(β= 0.3, SE= 0.2)	(β= 0.2, SE= 0.2)	(β= 0.2, SE= 0.2)	(β= 0.4, SE= 0.2)	(β= 0.3, SE= 0.2)
FTO	rs9939609	0.010	**0.004**	0.008	0.010	0.020
		(β= 0.6, SE= 0.2)	(β= 0.6, SE= 0.2)	(β= 0.6, SE= 0.2)	(β= 0.7, SE= 0.3)	(β= 0.7, SE= 0.3)
MC4R	rs17782313	0.377	0.726	0.243	0.094	0.328
		(β= 0.3, SE= 0.3)	(β= 0.1, SE= 0.3)	(β= 0.4, SE= 0.3)	(β= 0.6 , SE= 0.4)	(β= 0.4, SE= 0.4)
KCTD15	rs29941	0.301	0.192	0.319	0.177	0.405
		(β= 0.2, SE= 0.2)	(β= 0.2, SE= 0.2)	(β= 0.2, SE= 0.2)	(β= 0.3 , SE= 0.2)	(β= 0.2, SE= 0.3)

[1]P-values for association of obesity SNPs adjusted for age, sex, community group, and n-3 PUFA intake. Estimates of effect size (β) are reported for additive model and BMI-increasing alleles using transformed phenotypes.

[2]Results significant at $p \leq 0.005$ are highlighted in bold. Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

Table 4.7.5: Interaction with n-3 PUFA intake modifies the Genetic Risk Score association with adiposity phenotypes[1,2,]

Outcome	Main Effect[1]	Interaction[2]
BMI (kg/m^2)	**0.012**	**0.011**
Percentage Body Fat (%)	**0.022**	**0.025**
Hip Circumference (cm)	0.080	0.068
Thigh Circumference (cm)	**0.025**	0.084
Waist Circumference (cm)	**0.038**	**0.018**

[1] P-values for GRS analysis adjusted for sex, age, community group, and n-3 PUFA intake.

[2] P-values for interaction analysis (GRS*n-3 PUFA) adjusted for sex, age, community group and n-3 PUFA intake.

Table 4.7.6: Variation (%) in adiposity phenotypes explained by GRS association and GRS interactions with n-3 PUFA intake [1,2,3]

Variable	BMI	PBF	HC	ThC	WC
GRS	**0.7**	**0.3**	0.5	**0.7**	**0.5**
GRS*δ^{15}N	**1.7**	**0.8**	1.4	1.5	**1.4**

[1]Results significant at $p \leq 0.05$ are highlighted in bold.

[2]Variation attributed to GRS after adjustment for age, sex, community group, and n-3 PUFA intake.

[3]Variation attributed to GRS*n-3 PUFA interaction after adjustment for age, sex, community group and n-3 PUFA intake.

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4.9 APPENDIX

Supplementary Table 4.9.1: Skewness, kurtosis, and transformation information for adiposity phenotypes

	Untransformed		Transformed		
Variables	**Kurtosis**	**Skewness**	**Kurtosis**	**Skewness**	**Power Transformation**
BMI (kg/m^2)	5.08	1.16	2.69	0.13	-0.7
Percentage Body Fat (%)	2.12	0.17	2.13	0.00	0.75
Waist Circumference (cm)	3.80	0.82	2.66	0.04	-0.84
Hip Circumference (cm)	6.58	1.26	2.95	0.14	-2.19
Thigh Circumference (cm)	6.31	0.22	3.55	0.07	-0.3

[1] Skewness and kurtosis for obesity phenotypes were computed using R.

[2] Power transformation were computed using R.

Supplementary Table 4.9.2: Association of obesity SNPs with obesity phenotypes without $\delta^{15}N$ adjustment [1,2]

Gene	SNP	BMI	PBF	HC	ThC	WC
NEGR1	rs2815752	0.6607 (β=0.3, SE=0.2)	0.2671 (β=0.4 , SE= 0.2)	0.4394 (β=0.4 , SE= 0.2)	0.2953 (β=0.6 , SE= 0.3)	0.3526 (β=0.6 , SE= 0.3)
TMEM18	rs7561317	0.3414 (β=-0.4, SE= 0.2)	0.4273 (β=-0.3 , SE= 0.2)	0.5323 (β=-0.4 , SE= 0.2)	0.6557 (β=-0.4 , SE= 0.3)	0.2390 (β=-0.6 , SE= 0.3)
ETV5	rs7647305	0.0684 (β=1.5 , SE= 0.6)	0.0884 (β=1.3 , SE= 0.5)	0.0219 (β=1.8 , SE= 0.6)	0.0163 (β=2.2 , SE= 0.7)	0.0794 (β=1.9 , SE= 0.8)
GNPDA2	rs10938397	0.9880 (β=0.1 , SE= 0.2)	0.9820 (β=0.1 , SE= 0.2)	0.6998 (β=0.3 , SE= 0.2)	0.6224 (β=0.4 , SE= 0.3)	0.9866 (β=0.1 , SE= 0.3)
BDNF	rs6265	0.9641 (β=-0.3 , SE= 0.6)	0.9880 (β=-0.2 , SE= 0.5)	1.0000 (β=0.03 , SE= 0.6)	0.9999 (β=0.0 , SE= 0.7)	0.9204 (β=-0.6 , SE= 0.7)
MTCH2	rs10838738	0.9891 (β=-0.1 , SE= 0.2)	0.9649 (β=-0.1 , SE= 0.2)	0.9231 (β=0.1 , SE= 0.2)	0.8434 (β=0.2 , SE= 0.2)	0.9998 (β=0.0 , SE= 0.2)
SH2B1	rs7498665	0.4243 (β=-0.3 , SE= 0.2)	0.5391 (β=-0.2 , SE= 0.2)	0.6617 (β=-0.2 , SE= 02.)	0.4145 (β=-0.4 , SE= 0.2)	0.6373 (β=-0.3 , SE= 0.2)
FTO	rs9939609	0.0704 (β=0.6 , SE= 0.2)	0.0499 (β=0.6 , SE= 0.2)	0.0558 (β=0.6 , SE= 0.2)	0.0399 (β=0.8 , SE= 0.3)	0.1322 (β=0.7 , SE= 0.3)
MC4R	rs17782313	0.8323 (β=0.3 , SE= 0.3)	0.9883 (β=0.1 , SE= 0.3)	0.6604 (β=0.4 , SE= 0.3)	0.3373 (β=0.7 , SE= 0.4)	0.7588 (β=0.4 , SE= 0.3)
KCTD15	rs29941	0.8091 (β=0.2 , SE= 0.2)	0.6474 (β=0.2 , SE= 0.2)	0.8082 (β=0.2 , SE= 0.2)	0.5921 (β=0.1 , SE= 0.3)	0.8604 (β=0.3 , SE= 0.2)

[1]P-values for association of obesity SNPs adjusted for age, sex, community group. Estimates of effect size (β) are reported using transformed phenotypes.

[2]Results significant at $p \leq 0.005$ are highlighted in bold. Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

Supplementary Table 4.9.3: Interaction between n-3 PUFA intake and obesity SNPs modifies association with obesity traits[1,2]

Gene	SNP	BMI	PBF	HC	ThC	WC
NEGR1	rs2815752	0.4367	0.3861	0.0544	0.1084	0.1944
TMEM18	rs7561317	0.0249	0.1545	0.1961	0.2057	0.0389
ETV5	rs7647305	0.0243	0.0243	0.0092	0.0127	0.0283
GNPDA2	rs10938397	0.4953	0.2321	0.2648	0.5940	0.2610
BDNF	rs6265	0.7193	0.9837	0.8573	0.7912	0.6430
MTCH2	rs10838738	0.6633	0.6681	0.2420	0.3675	0.2571
SH2B1	rs7498665	0.3626	0.4967	0.6919	0.5326	0.3517
FTO	rs9939609	0.0516	0.0080	0.0140	0.0293	0.0238
MC4R	rs17782313	0.3179	0.6694	0.3265	0.2669	0.3837
KCTD15	rs29941	0.6724	0.1702	0.5267	0.5932	0.8650

[1]P-values for interaction analysis (SNP*n-3 PUFA) adjusted for age, sex, community group, and n-3 PUFA intake.

[2]Results significant at $p \leq 0.005$ are highlighted in bold. Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC) and waist circumference (WC).

Supplementary Table 4.9.4: Interaction with n-3 PUFA intake modifies the genetic risk score (GRS) association with obesity phenotypes after correction for intake of total fat and total energy (n=492)

Outcome	Total Fat (%)[1]	Total Energy (kcal)[2]
BMI (kg/m^2)	**0.034**	**0.033**
Percentage Body Fat (%)	0.228	0.180
Hip Circumference (cm)	0.347	0.340
Thigh Circumference (cm)	0.134	0.079
Waist Circumference (cm)	0.056	**0.038**

[1]P-values for GRS*n-3 PUFA analysis adjusted for sex, age, community group and total fat intake (%).

[2]P-values for GRS*n-3 PUFA analysis adjusted for sex, age, community group and total energy intake (kcal).

SUMMARY AND FUTURE DIRECTIONS

5.1 DISSERTATION OBJECTIVES

Obesity is a highly heritable trait that ultimately increases the risk of developing T2D, CVD, and other diseases (1). Although molecular approaches have expedited the discovery of human obesity genes, the heritable component of obesity explained by these genes remains low (<2%) (2). In parallel to these observations, accumulating evidence show n-3 PUFA intake has anti-obesity effects (3), yet the extent to which n-3 PUFA intake modulates the genetic risk of obesity is unknown.

Increasingly, isolated populations have been shown to facilitate the discovery of genetic and environmental factors underlying variation in complex phenotypes (4). Isolated populations are homogeneous sub-groups of individuals that are often characterized by a shared environment and geographic isolation over long periods of time (5). Candidate gene studies have benefited from sampling genetically isolated populations, in part, because large families that share a uniform environment are expected to increase the power of detecting genetic factors associated with complex phenotypes such as obesity (6). The dissertation analyzed data from the CANHR study (7), a geographically isolated cohort of Yup'ik participants that belong to large extended families, engage in traditional subsistence activities and have widely varying consumption of n-3 PUFAs (8). Prevalence of T2D is currently low in this population however the prevalence of T2D among Yup'ik people is reported to be rising rapidly (9), and a better understanding of obesity and its determinants will help in developing interventions effective for this population.

The CANHR study is ideally suited to investigate the contribution of n-3 PUFA intake and genetic factors to variation in obesity phenotypes due to the 30-fold range of n-3 PUFA consumption (10) that can be precisely estimated in large samples using a validated biomarker (11). Moreover, our studies provide an opportunity to directly investigate the health benefits of a subsistence diet that is rich in PUFAs and has great

cultural importance to Yup'ik people. Specifically, we assessed the differences in n-3 PUFA intake among CANHR study participants using nitrogen stable isotope ratios $\delta^{15}N$ as an objectively measured biomarker of n-3 PUFA intake (11). Estimating n-3 PUFA intake using $\delta^{15}N$ should provide us with an integrated signal of n-3 PUFA intake that could modulate candidate gene expression over a 3-4 month period (range of RBC lifetime is approximately 120 days). Finally, this dissertation benefited from a large cohort of Yup'ik participants and statistical methodologies that account for large-extended families (6).

Thus, the overall objective of this work was to examine gene-diet interactions in a study population with widely varying n-3 PUFA intake: to evaluate the extent to which genetic polymorphisms are associated with obesity-related phenotypes in Yup'ik people, and assess how these associations may be modified by n-3 PUFA intake. The selected candidate genes presented in this dissertation included: (1) candidate genes with a strong physiological role in obesity pathophysiology; (2) candidate genes identified in obesity whole-genome linkage studies that are also regulated by n-3 PUFAs; and (3) candidate genes reproducibly implicated in obesity genome-wide association studies. In the following sections, I outline the objectives of each study and summarize our major findings.

5.2 DISSERTATION SUMMARY

5.2.1 PHYSIOLOGY

We selected carnitine palmitoyltransferase 1A (*CPT1A*) as a strong physiologocal candidate gene that is implicated in obesity pathophysiology. CPT1A controls fatty acid oxidation in liver tissue (12) and facilitates the transfer of long-chain fatty acids (LCFA) across the mitochondrial membrane for β-oxidation (13). A meta-analysis of whole-genome linkage studies has implicated *CPT1A* as an obesity candidate gene (14) and the P479L variant of *CPT1A* polymorphism has been associated with elevated fasting HDL-cholesterol and ApoA1 levels in Greenland Eskimos (15). Given that consumption of n-3

PUFA increases the activity of CPT1A (16) and functional evidence demonstrates L479 allele of P479L both reduces CPT1A activity (~80%) and abolishes CPT1A inhibition by Malonyl CoA (17); this study examined 28 tagging *CPT1A* SNPs, including the P479L polymorphism, for association with obesity-related phenotypes in Yup'ik people and explored whether these associations were modified by n-3 PUFA intake.

Our results demonstrate the P479L (rs80356779) polymorphism in *CPT1A* is negatively associated with all obesity phenotypes in Yup'ik people and we also replicated the positive association of P479L with fasting HDL-cholesterol. Specifically, we found that individuals homozygous for the L479 allele have a lower percentage body fat, smaller BMI, and reduced thigh, hip, and waist circumferences compared to P479 homozygotes. Furthermore, our analyses indicate the P479L association with fasting HDL-cholesterol was still significant after correcting for BMI, percent body fat (PBF), or waist circumference (WC). Taken together, our results suggest that there will be net increase in the basal activity of CPT1A among individuals carrying the L479 allele and the P479L variant in Eskimo/Inuit populations with elevated consumption of n-3 PUFAs, and this may be cardio-protective (through increased HDL-cholesterol) and reduced adiposity.

5.2.3 WHOLE-GENOME LINKAGE STUDIES

We selected *SCD*, *SLC2A4*, and *SREBF1* as candidate genes for this study based on evidence from obesity whole-genome linkage analyses (18–20) and functional studies indicating that *SCD* gene expression (21), *SLC2A4*-mediated glucose transport (22), and *SREBF1* gene transcription (23) are regulated by n-3 PUFA intake. Previous candidate gene studies have demonstrated that *SCD*, *SLC2A4*, and *SREBF1* polymorphisms are associated with obesity-related traits (24–28), however the extent to which n-3 PUFA intake modifies these genetic associations remains unknown. Our results demonstrate that in Yup'ik people, *SCD* polymorphisms (rs1190480 and rs2167444) are associated with reduced BMI, smaller hip circumference and elevated fasting ApoA1 levels, the

major apolipoprotein found in HDL-cholesterol. Furthermore, *SLC2A4* polymorphisms are associated with increased adiposity and lower fasting HDL-cholesterol levels. In light of functional evidence that demonstrates n-3 PUFA intake transcriptionally regulates *SCD* (21) and *SLC2A4* (22), we did not find that consumption of n-3 PUFA intake modified *SCD* and *SLC2A4* polymorphisms associated with obesity phenotypes in this Yup'ik study population. We did observe *SCD* polymorphisms (rs1190480 and rs2167444) associated with fasting total cholesterol levels that were modified by n-3 PUFA intake; however these gene-diet interactions were only nominally significant after adjusting for HDL-cholesterol. Taken together, these results suggest the interaction of *SCD* polymorphisms and n-3 PUFA intake on total cholesterol levels may, in part, be mediated by changes in fasting HDL-cholesterol levels.

5.2.4 GENOME-WIDE ASSOCIATION STUDIES

We selected *NEGR1, TMEM18, ETV5, GNPDA2, BDNF, MTCH2, SH2B1, FTO, MC4R* and *KCD15* as obesity candidate genes based on evidence that these genes were reproducibly implicated in obesity GWAS. Previous candidate genes studies have largely replicated the association of obesity GWAS polymorphisms in diverse populations (29); however, the extent to which obesity GWAS polymorphisms are associated with obesity phenotypes in Yup'ik people and how these polymorphisms interact with modifiable environmental factors such as n-3 PUFA intake was lacking. Our results demonstrate that the rs9939609 SNP in the *FTO* gene was positively associated with percent body fat and supports other studies indicating that variation in *FTO* contributes to fat accumulation (30). Interestingly, we found that the rs7647305 SNP in the *ETV5* gene was associated with adiposity phenotypes in our study; however, the direction of the association was the reverse of that reported in participants of Europeans ancestry (31, 32). Although our results in this Yup'ik study population are "opposite" to those observed in Europeans, theoretical modeling has argued that "flip-flop" associations can be attributed to population specific differences in linkage

disequilibrium patterns among SNPS in a particular genomic region (33).

We estimated the cumulative effects of obesity GWAS polymorphisms to changes in adiposity phenotypes in Yup'ik people using a genetic risk score (GRS) for obesity. An obesity GRS is designed to estimate an individual's genetic predisposition to obesity by adding the BMI-increasing alleles across all the obesity SNPs of interest. We found the GRS was positively associated with adiposity phenotypes, and this association was modified by n-3 PUFA intake. Specifically, genetic predisposition to obesity, as measured by the GRS, was positively associated with adiposity and n-3 PUFA intake modified these associations whereby among individuals with a low GRS, n-3 PUFA intake did not increase BMI. However, in individuals with a moderate (2) or high (3) GRS, n-3 PUFAs intake is obesigenic. Taken together, these results suggest the anti-obesity effects of n-3 PUFA in humans may be dependent upon an individual's genetic predisposition to obesity.

Our analysis that examined the extent to which gene-by-diet interactions explained the heritable component of obesity revealed the interactions of GRS and n-3 PUFA intake on adiposity accounted for more than twice the phenotypic variation (~2% variation explained), relative to GRS associations alone (~1% variation explained). Although modest, our results support the findings of Li *et al.* (34) that demonstrated physical activity interactions with a GRS, calculated from 12 obesity risk alleles, accounted for twice as much phenotypic variation in obesity-related traits relative to the genetic risk score alone (34). Taken together, this study provides evidence that interactions between n-3 PUFA intake and obesity GWAS polymorphisms may account for a small portion of the "missing heritability" attributed to obesity. Furthermore, the results of this study extends what is known about n-3 PUFAs by demonstrating the anti-obesity effects of n-3 PUFA intake may be dependent upon an individual's genetic predisposition to obesity.

5.3 CONCLUSIONS

Advanced molecular approaches such as high-throughput genotyping and whole-genome sequencing have expedited the discovery of human obesity genes, however the heritable component of obesity explained by the aggregate of these loci remains low (<2%) (2). Evidence that obesity occurs in response to interactions between genetic and environmental factors may help account for the "missing heritability" attributed to obesity and inform personalized interventions. In this dissertation, we examined (1) candidate genes with a strong physiological role in obesity pathophysiology; (2) candidate genes identified in obesity whole-genome linkage studies that were regulated by n-3 PUFAs; and (3) candidate genes reproducibly implicated in obesity GWAS. Our investigation of candidate genes based on physiology demonstrates the non-synonymous polymorphisms called P479L in *CPT1A* was associated with elevated fasting HDL-cholesterol and all obesity phenotypes. Our investigation of candidate genes that are regulated by n-3 PUFAs and implicated in obesity whole-genome linkage studies demonstrate that polymorphisms in *SCD* and *SLC2A4* were associated with obesity-related phenotypes and n-3 PUFA intake modified the *SCD* polymorphisms associated with fasting cholesterol levels. Finally, our investigation of candidate genes reproducibly implicated in obesity GWAS demonstrated that genetic predisposition to obesity, estimated using a GRS, is associated with adiposity and that interactions with n-3 PUFA intake accounted for more than twice the phenotypic variation in adiposity. Taken together, this dissertation suggests that selecting candidate genes based on large-scale genomic dataset, such as linkage analyses and GWAS, has the potential to identify gene-by-environment interactions that partially account for the "missing heritability" attributed to obesity.

5.5 FUTURE DIRECTIONS

Existing epidemiological approaches have elucidated genetic and environmental risk factors that increase the risk of developing obesity; however mechanistic information

that accounts for these observations is limited. Epigenetics describes the phenomena where inherited alterations in gene function occur independently of changes in the nucleotide sequence (35). In the case of obesity, epigenetic modifications (DNA methylation and histone modification) are associated with changes in body weight among genetically identical mice (36). Interestingly, diet and physical activity have been shown to impact epigenetic modification (37) and this observation generates an intriguing hypothesis that epigenetic processes are actively influenced by lifestyle (38). Taken together, future directions should consider epigenetic modification as a putative mechanism explaining how specific environmental exposures can impact obesity independent of genetic variation (39).

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APPENDIX

Appendix 6.1 Alaska Area Institutional Review Board

4315 Diplomacy Drive
Anchorage, Alaska 99508
(907) 563-2662

October 28, 2003

Gerald Mohatt, PH.D.
Center for Alaska Native Health Research
Institute of Arctic Biology
University of Alaska Fairbanks
311 Irving 1, Box 757000
Fairbanks, Alaska 99775-7000

Dear Dr. Mohatt:

The responses to our comments on your last submission for proposal **03-P-15 "A Study of Obesity and Diabetes among Yup'ik Eskimos"** .were considered adequate with the following clarifications:

- You are required to explicitly state that when participants turn 18 years of age the parental consent that was received for their participation is no longer valid. Analysis of previously obtained data may occur, but no further information may be collected from those persons unless individual adult consent is obtained.
- Your most recent response did not include any mention of the specimen banking protocol. We require that you agree to abide by the currently approved Alaska Area/CDC serum bank.

You are again commended for your prompt and thorough attention to our previous comments. If the above conditions are met, the project is approved for one year. You are required to fulfill the education requirement by having all personnel involved in a substantive way in the research project complete the website training at www.miami.edu/citireo if you have not done so, the instructions are attached.

Any manuscripts or reports for publication, or abstracts for meeting presentation must be submitted to Dr. Ruth Etzel and Dr. Anne Lanier for review PRIOR TO SUBMITTAL. Please submit copies of the final approval versions to the IRB for your protocol file. Please be advised that research involving Alaska Native Tribal Health Consortium must be reviewed by Dr. Ruth Etzel, Southcentral Foundation and Dr. Anne Lanier, ANTHC. Please e-mail your proposal to retzel@anmc.org and alanier@anthc.org.

"This IRB action does not constitute review or compliance with HIPAA requirements. Prior to access and/or use of data, you must receive approval from the appropriate institutional officials releasing this information under the current HIPAA requirements."

You can write to Jennifer at jali@anmc.org at the ANMC address, or call her at (907) 729-2061 between the hours of 8:00 am and 4:30 pm, Monday through Friday for any questions you might have. Thank you.

Sincerely,

Terry J. M. Powell
Deputy Chairperson
Alaska Area IRB

cc: 03-P-15

Appendix 6.2 Indian Health Service Institutional Review Board

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Indian Health Service
Rockville MD 20852

SEP 16 2003

Gerald V. Mohatt, EdD
Director, Center for Alaska Native Health Research
Irving I, room 311
P.O. Box 757000
Fairbanks, Alaska 99775

RE: Research Protocol 9AK03 **"A study of Obesity and Diabetes among Yup'ik Eskimos"**

Dear Mr. Mohatt:

The Indian Health Service (IHS) National Institutional Review Board (IRB) has reviewed your protocol entitled, **"A study of Obesity and Diabetes among Yup'ik Eskimos** The IRB **approved** your protocol.

The approval is good for one year, from the date of this letter and will require continuation approval if the research project extends beyond September 15, 2004. The project is approved to proceed with the understanding that it will adhere to the Health Insurance Portability and Accountability Act (HIPAA) guidelines, which it currently meets as proposed. Please remember that any major changes can not be made to the research protocol without receiving prior approval from the National IHS IRB.

If you have any questions about the IRB's decision, please contact me at (301) 443-6528, or by e-mail at Psmith@hqe.ihs.gov. Thank you for submitting this research proposal to the National IHS IRB. We appreciate your interest in providing the benefits of health research to American Indian and Alaskan Native individuals and communities.

Sincerely,

Phillip Smith, MD, MPH
Chair, National IHS IRB
MPA – 1493

cc: Dave Barrett, Chair, Alaska IHS IRB
Terry Powell, Co-Chair, Alaska IHS IRB
file

Appendix 6.3 Yukon-Kuskokwim Health Corporation Institutional Review Board

YUKON-KUSKOKWIM HEALTH CORPORATION

"Fostering Native Self-Determination in Primary Care, Prevention and Health Promotion."

March 8, 2004

Gerald Mohatt, EdD,
Irving I. Room 311
P.O. Box 757000
Fairbanks, Alaska 99775

Dear Mr. Mohatt:

This letter is confirmation that on October 22, 2003, the Yukon Kuskokwim Health Corporation's Executive Board approved the CANHR study proposal. For YKHC tracking purposes it has been labeled **02.04.09 CANHR: A Study of Obesity and Diabetes Among Yup'ik Eskimos.** Please reference this title and YKHC number in all correspondence regarding this study.

These special stipulations for the CANHR protocol were requested.

1. Change consent check boxes to initial boxes.
2. Add to F in consent form "used in future by YKHC Board approval for genetic and medical studies."
3. For H- change Bethel to YK region
4. The Dietary Intake form has both participant ID, name, and DOB. Do not collect name only ID number.
5. Add in consent non-identifier raw data may be used in conjunction with EARTH study.
6. Obtain YK Release of Information form if chart reviews are anticipated.

It is expected that annual updates will be submitted in an electronic format until such time the study is completed. A copy of the study update format is being attached to the email version of this confirmation.

This approval is valid for one year and will be updated annually. Notification of any adverse events should be reported to my office immediately. Formal YKHC approval is required for any abstracts, papers, or publications arising from YKHC approved research (see process below).

YKHC supports the work you are doing in increasing the knowledge of best health practices for our population through your research. We anticipate that your work will

lead to formal publications in the future. To assist in timely approval of future manuscripts, please plan on providing YKHC at least a two-month window to review and approve any such manuscripts. The YKHC Executive Board meets every other month and depending on time of submission, approval could take even longer since time must be allowed for the YKHC Human Studies Committee to review the manuscript and then recommend it to the YKHC Board for approval. For best results we would suggest beginning communications with my office as early as possible to help coordinate timing of submittal for review so as to assist in a quick approval process. Also keep in mind that if village traditional council approval was required for this study, that annual updates should be made to the village(s). Approval for publications may also need to be obtained from such villages.

When submitting manuscripts for approval, please provide:

1. An electronic copy of the full manuscript (email to joe_klejka@ykhc.org)
2. Complete the YKHC manuscript info sheet (attached to email copy of this letter) and submit electronically as well.
3. Provide designated fee to offset costs incurred by YKHC in review process

Once again, YKHC sincerely appreciates your work, enthusiasm, and effort, and looks forward to your continued success.

Sincerely,

Joseph Klejka, MD
YKHC Corporate Medical Director

June 4, 2007

Dr. Bert Boyers
Center for Alaska Native Health Research
311, Irving I Bldg
Fairbanks, Alaska 99775

Dear Dr. Boyer:

This letter is confirmation that on April 25, 2007, the Yukon Kuskokwim Health Corporation's Full Board of Director's approved the proposal for the **Genetics of Obesity in Yup'ik Eskimos** study. For YKHC tracking purposes it has been labeled **07.02.01 Genetic of Obesity in Yup'ik Eskimos.** Please reference this title and YKHC number in all correspondence regarding this study.

Special stipulations for the **07.02.01 Genetic of Obesity in Yup'ik Eskimos** were requested prior to conducting the study.

1) Rephrase statement regarding DNA ownership (review with Aileen Havilland and myself).
2) Assure that Release of Information (ROI) is obtained from participants if medical record review is to be performed.
3) Correct the discrepancy between the protocol and the consent form regarding native blood quantum.
4) On the consent form, remove the phrase stating that treatment is available at no cost.
5) Correct statement that 26cc is less than a tablespoon.
6) Changes needed regarding item E on consent form regarding banking of blood specimens (obtain appropriate language from Aileen Havilland and myself).
7) To mitigate exercise-testing risk, please add to your protocol a screen for resting pulse (needs to be less than 100) and resting blood pressure (needs to be less than 160/100) prior to exercise testing.

It is expected that annual updates will be submitted in an electronic format until such time the study is completed. A copy of the study update format is being attached to the email version of this confirmation.

This approval is valid for one year and will be updated annually. Notification of any adverse events should be reported to my office immediately. Formal YKHC approval is required for any abstracts, papers, or publications arising from YKHC approved research (see process below).

YKHC supports the work you are doing in increasing the knowledge of best health practices for our population through your research. We anticipate that your work will lead to formal publications in the future. To assist in timely approval of future manuscripts, please plan on providing YKHC at least a two-month window to review and approve any such manuscripts. The YKHC Executive Board meets every other month and depending on time of submission, approval could take even longer since time must be allowed for the YKHC Human Studies Committee to review the manuscript and then recommend it to the YKHC Board for approval. For best results we would suggest beginning communications with my office as early as possible to help coordinate timing of submittal for review so as to assist in a quick approval process. Also keep in mind that if village traditional council approval was required for this study, that annual updates should be made to the village(s). Approval for publications may also need to be obtained from such villages.

When submitting manuscripts for approval, please provide:

1. An electronic copy of the full manuscript (email to joseph_klejka@ykhc.org)
2. Complete the YKHC manuscript info sheet (attached to email copy of this letter) and submit electronically as well.
3. Provide designated fee to offset costs incurred by YKHC in review process

Once again, YKHC sincerely appreciates your work, enthusiasm, and effort, and looks forward to your continued success.

Sincerely,

Joseph Klejka, MD
YKHC Corporate Medical Director
907-543-6028 or 6027
fax 907-543-6091 or 6006

Appendix 6.4 University of Alaska Fairbanks Institutional Review Board

University of Alaska Fairbanks

INSTITUTIONAL REVIEW BOARD

Karin Davidson
Research Committee Coordinator
Office of Research Integrity
University of Alaska Fairbanks
206 Elelson Building, P.O. Box 757560
Fairbanks, AK 99775-7560

(907) 474-7800
email: k.davidson@ uaf.edu

April 30, 2003

Subject: IRB review of Human Subjects Application form IRB #03-16

Dear Dr. Mohatt:

The following Human Subjects Application was reviewed by the University of Alaska Fairbanks Institutional Review Board (IRB) at the March 27, 2003, March 28, 2003 and April 24, 2003 meetings:

IRB Protocol Number:	#03-14
Investigator/Instructor:	Gerald Mohatt
Title of Project/Course:	*Center for Alaska Native Health Research* (COBRE Grant 1 P20 RR16430-01): *Obesity, cardiovascular disease, and diabetes among Yup'ik Eskimos*
Date Received:	March 17, 2003
Date Approved:	April 24, 2003
Annual Renewal:	Due April 2004 (Annual Renewal is required)

Minority Opinions:
None.

Procedural changes or amendments must be reported to the IRB, and no changes may be implemented without prior IRB approval.

Karin Davidson
Research Committee Coordinator

UAF
UNIVERSITY OF ALASKA FAIRBANKS

(907) 474-7800
(907) 474-5444 fax
fyirb@uaf.edu
www.uaf.edu/irb

Institutional Review Board
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

December 8, 2006

To: Bert Boyer, PhD
Principal Investigator

From: Bridget Stockdale, Research Integrity Administrator
Office of Research Integrity

Re: IRB Protocol Application

The University of Alaska Fairbanks' Institutional Review Board (IRB) tabled the following protocol at their November 1, 2006 meeting until the requested clarifications/modifications were received. The revised protocol and forms received on November 29, 2006 were reviewed at the December 6, 2006 meeting and the board voted to approve them by the following vote: 5 for, 0 against, 0 abstain, and 4 absent. Therefore, on behalf of the IRB I am pleased to issue final approval for this protocol.

Protocol#:	06-72
Title:	*Genetics of Obesity in Yup'ik Eskimos*
Received:	October 5, 2006 (orig) November 29, 2006 (rev)
Approved:	December 6, 2006

Any modification or change to this protocol must be approved by the IRB prior to implementation. Modification Request Forms are available on the IRB website (http://www.uaf.edu/irb/Forms.htm). Please contact the Office of Research Integrity if you have any questions regarding IRB policies or procedures.

UNIVERSITY OF ALASKA FAIRBANKS

Institutional Review Board

CONTINUING REVIEW REPORT

Principal Investigator: Bert Boyer, PhD Phone: 474-7733 e-mail: bboyer@alaska.edu
Graduate Student: Phone: e-mail:
Department: Institute of Arctic Biology
Project Title: Genetics of Obesity in Yup'ik Eskimos
CR Submission Date: 12/9/08
Protocol IRB #: 06-72 Approval Date: Review Level:

COMPLETE ALL ITEMS.

1. Please check as appropriate:
   - ☐ Project Completed.
   - ☒ Renew IRB Protocol for another year.
   - ☐ Project not funded. Withdraw IRB Protocol.
2. Maximum number of people approved for inclusion in this study: 1,000
3. Total number of people enrolled in this protocol to date: 485
4. Are new people continuing to be enrolled in this protocol? ☒ Yes ☐ No
5. Is data collection ongoing with currently enrolled individuals? ☐ Yes ☒ No
6. Have any unexpected risks or problems been encountered since the last review? ☐ Yes ☒ No
   If yes, describe the risks or problems. (attach additional information if necessary)
7. Were any individuals withdrawn from this study? ☒ Yes ☐ No
   If yes, describe reasons for withdrawal. (attach additional information, if necessary)
   Two participants withdrew because of pregnancy and four others withdrew due to time constraints.
8. Have any grievances/complaints been received since the last review of this study? ☐ Yes ☒ No
   If yes, describe grievance or complaint. (attach additional information, if necessary)
9. What is the current disposition (means of disposal or storage location) of collected human biological specimens?
   All samples are stored in a locked -80C freezer in room 250 WRRB (plasma, serum, RBC's) or in a locked -80C frezer in the Freezer Storage Room 244 AHRB. Hair samples are stored in Room 216 AHRB or in Diane O'Brien's lab Room 221.
10. Provide a summary of advances in this field since the last review of this protocol. Provide no more that five relevant citations from peer-reviewed literature.
    The biggest advances in the field of obesity genetics have come from Genome Wide Association Studies (GWAS) where typically 500,000 - 1,000,000 single nucleotide polymorphisms (SNPs) are genotyped in 5,000-20,000 unrelated individuals. The SNPs are evenly distributed throughout the genome and when one finds a significant association between a SNP and obesity phenotype, it suggests that the gene in which the SNP resides, may influence the development of obesity. Several GWAS have been completed and this presents an opportunity to more closely scrutinize the genes of interest, replicate the associations in other population groups, and to investigate gene by environment interactions that may modify the association between the SNP and obesity phenotype. We are investigating the association of SNPs in candidate genes for obesity (identified in GWAS and linkage studies), and

also looking at gene by environment interactions. The two main environmental modifiers we are studying are physical activity and polyunsaturated fatty acids in the diet. Here are 5 relevant citations: 1. Andreasen CH, Andersen G. Gene-environment interactions and obesity—further aspects of genomewide association studies. Nutrition. 2009 Oct;25(10):998-1003. Epub 2009 Jul 12. Review. PubMed PMID: 19596186. 2. Shuldiner AR. Obesity genes and gene-environment-behavior interactions: recommendations for a way forward. Obesity (Silver Spring). 2008 Dec;16 Suppl 3:S79-81. PubMed PMID: 19037219; PubMed Central PMCID: PMC2703439. 3. Heard-Costa NL, Zillikens MC, Monda KL, Johansson A, et al. NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. PLoS Genet. 2009 Jun;5(6):e1000539. Epub 2009 Jun 26. PubMed PMID: 19557197; PubMed Central PMCID: PMC2695005. 4. Lindgren CM, Heid IM, Randall JC, Lamina C, et al. Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet. 2009 Jun;5(6):e1000508. Epub 2009 Jun 26. Erratum in: PLoS Genet. 2009 Jul;5(7). doi: 10.1371/annotation/b6c8f9f6-2496-4a40-b0e3-e1d1390c1928. PubMed PMID: 19557161;PubMed Central PMCID: PMC2695778. 5. Willer CJ, Speliotes EK, Loos RJ, Li S, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet. 2009 Jan;41(1):25-34. Epub 2008 Dec 14. PubMed PMID: 19079261; PubMed Central PMCID: PMC2695662.

11. Summarize your interim findings, and any amendments or modifications to the research since the last review.

The following modifications have been submitted and approved since the last review approved 11/18/09:

1. Approved February 25, 2009 - Addition of Salena Bias, Student employee in CANHR EB Core.

2. Submitted September 20, 2009 and currently under review - a) To add Marjorie Richards, Research Nurse, and Anna Peter-Raboff, Translator to the study protocol; b) To include the measurement of stable isotope ratios (13C/12C, 15N/14N, 35S/34S, D/H, and 18O/16O) in blood samples of study participants.

3. Submitting modification on December 9, 2009 with this review - request to modify consent forms (see attached modification request with consents).

12. What type of funding supports this project?

☐ Internal Source:
☒ External UAF Proposal or Grant # 7997 Agency: NIH R01 DK074842
☐ Unfunded

13. Provide a list of all personnel currently working on this protocol:

Name	Email Address *UAF email (ex: ffabc1) required for faculty, staff and students*
Scarlett Hopkins	sehopkins2@alaska.edu
Jynene Black	jablack@alaska.edu
Eliza Orr	emorr@alaska.edu
Dominick Lemas	dlemas@alaska.edu
Maria Bray	mdbray@alaska.edu
Peggy Williams, Field Research Asst. Henry Lupie, Field Research Asst. Paul Andrew, Field Research Asst.	
Fancy Lomack, Field Research Asst. Filma Peter, Field Research Asst.	
Anna Angaiak, Field Research Asst. Katherine Charles, Field Research Asst.	
Marjorie Richards, Research Nurse	marichards@alaska.edu
Anna Peter-Raboff, Translator	apeterraboff@alaska.edu
Salena Bias	skbias@alaska.edu
Maria Anastario	manastario@alaska.edu

14. Please check all that are applicable:

☒ Written **consent** forms approved by the UAF IRB are used with this protocol.
Attach a copy of the current form(s)

☒ Written **parental consent** forms approved by the UAF IRB are used with this protocol.
Attach a copy of the current form(s)

☒ Written **child assent** forms approved by the UAF IRB are used with this protocol.
Attach a copy of the current form(s)

PRINCIPAL INVESTIGATOR DECLARATION: On behalf of my co-investigators, associated students, staff and myself, I agree: To perform the research according to UAF policy and the underpinning ethical principles on which it is based; to strictly adhere to the research protocol as it relates to human subjects, and to ensure that no changes will be made in the activity without obtaining prior IRB approval (except that a change may be made to eliminate apparent immediate hazards to the subject); to comply with any contingencies upon which approval may be granted; to promptly notify the Office of Research Integrity verbally (with written confirmation following) of unanticipated problems involving risk to subjects or others and of any other adverse circumstances or reactions affecting the subjects that arise from the research. ***Note: The IRB no longer requires the PI's signature on Continuing Review Reports provided the form is submitted electronically by the PI (i.e. from the PI's UAF email account).***